Review of Literature
II. REVIEW OF LITERATURE

In this chapter, an effort has been made to present the existing literature on the importance, origin, distribution, taxonomy, classification, descriptive studies, evolutionary aspects and morphological studies on different Jasmine species and varieties. The work done on other related ornamental crops are also included as the information available on the use of molecular markers for discriminating cultivars, genetic purity, genetic diversity, phylogenetic relationship and crop improvement in jasmine is scanty.

2.1 Importance

Jasmine is an important traditional flower crop of our country. A number of varieties of a few Jasmine species are grown all over the Karnataka state. The important jasmine cultivars grown in the state are Gundu mallige \((Jasminum sambac Ait)\), Jaji Mallige \([Jasminum grandiflorum (Linn).]\), Kakada \([Jasminum multiflorum (Burm.F.)]\) and Sooji Mallige \((Jasminum auriculatum Vanl.)\).

Jasmine is grown for its highly scented flowers. The flower buds are harvested every day morning and are tied together along a single plane using banana fiber and thread. A group of about two hundred flower buds tied together is called an ‘Atte’ and one long ‘Atte’ consists of about eight hundred flower buds folded into four rows and is called as ‘Chendu’. The flowers are used for hair adornments as Veni, and also in performing pooja. The flowers usually last for a day and the buds usually open between 6.30 to 7.30 pm. The flowers have a very good demand in local market and a considerable amount of flowers are marketed to Mumbai and also exported to Gulf countries. They also have industrial uses viz. essential oil extraction and cosmetic preparations.

2.2 Origin and distribution

Jasmines are native to tropical and subtropical regions of the world. They are native to Indo-Malaya region (Anonymous, 1959) and belong to the family Oleaceae. The word ‘Jasmine’ is said to have been derived from old Persian name of ‘Yasmyn’ meaning fragrance (Bhattacharjee, 1980). The genus was originally reported to
comprise about 200 species (Rendle, 1925; Bailey, 1958). However, Veluswamy et al. (1975) suggested that the number of true species is lesser (only to be 89). Similar, lesser number of species in *Jasminum* is also reported by Cooke (1905). The distribution patterns of nearly 72 species in India, Malaysia and China provide a strong base to claim that India is one of the principal centers of origin of *Jasminum* species. Among these, about 40 species are reported to occur in India. Large number of species are however, centered to the region comprising of The Himalayas, China and Malaysia (Veluswamy et al., 1975). Other countries where jasmine is cultivated are France, Italy, Morocco, Algeria, North Africa, Spain and Egypt. The cultivated jasmines fall into four species viz., *Jasminum sambac*, *Jasminum auriculatum*, *Jasminum grandiflorum*, and *Jasminum multiflorum* (Kakada).

In India, the three species *Jasminum sambac*, *Jasminum auriculatum* and *Jasminum grandiflorum* have been mentioned in the ancient Tamil Literature (500 B.C to 200 A.D). India could have been an important center of origin for many of the *Jasminum* species (Veluswamy et al., 1975). At present, Karnataka, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Bihar and West Bengal are the important jasmine growing states.

### 2.3. Taxonomy

#### 2.3.1. Classification and descriptive studies in *Jasminum* species

Walpers (1852) classified *Jasminum* into two sections based on leaf lets and further classified the sections into sub sections based on calyx characteristics.

<table>
<thead>
<tr>
<th>Section I – Unifoliata</th>
<th>Section II – Trifoliata</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Calyx: Lobes subulate and elongate</td>
<td>a. Calyx: Shortly dentate</td>
</tr>
<tr>
<td>b. Calyx: Sub-truncate</td>
<td>b. Calyx: Lacinate</td>
</tr>
</tbody>
</table>

One of the earliest accounts of descriptive studies in 43 jasmine species was accomplished by Hooker (1882) indicating two main groups of *Jasminum* as indicated below

- **Group I** - with simple leaves, calyx pubescent / glabrous, subulate / short
- **Group II** - with compound leaves either of trifoliate or imparipinnate
Engler and Prantl (1897) cited about 160 species of jasmine in the tropical and subtropical regions of Asia, Africa, Australia and over 40 in India. They considered that the simple leaf was only the transformed terminal leaflet of the imparipinnately compound leaf. They grouped jasmine into four sections:

<table>
<thead>
<tr>
<th>Section I - Unifoliata</th>
<th>Section II - Trifoliata</th>
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</thead>
<tbody>
<tr>
<td>Section III - Alternifolia</td>
<td>Section IV - Pinnatifolia</td>
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</table>

In India, Gamble (1936) published descriptions on 20 species occurring in Presidency of Madras and classified them based on Hooker’s classification (leaf characters).

<table>
<thead>
<tr>
<th>Leaf characteristics</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group-I: Simple leaf type</strong></td>
<td></td>
</tr>
<tr>
<td>Calyx pubescent, subulate and long</td>
<td><em>Jasminum sambac</em> and <em>Jasminum pubescens</em></td>
</tr>
<tr>
<td>Calyx pubescent, linear and short</td>
<td><em>Jasminum arborescens</em></td>
</tr>
<tr>
<td>Under calyx glabrous or nearly so, linear and long</td>
<td><em>Jasminum rigidum</em></td>
</tr>
<tr>
<td><strong>Group-II: Compound Leaf type</strong></td>
<td></td>
</tr>
<tr>
<td>Leaves trifoliate, lower leaflets wanting and calyx lobes small</td>
<td><em>Jasminum auriculatum</em></td>
</tr>
<tr>
<td>Leaves trifoliate, lateral leaves little smaller than terminal and calyx lobes minute</td>
<td><em>Jasminum flexile</em> and <em>Jasminum calophyllum</em></td>
</tr>
<tr>
<td>Leaves imparipinnate and opposite</td>
<td><em>Jasminum grandiflorum</em></td>
</tr>
</tbody>
</table>

Taxonomic descriptions of six species were made by Bor and Raizada (1946) and four species viz., *Jasminum sambac*, *Jasminum pubescens*, *Jasminum primuliflorum* and *Jasminum grandiflorum* were considered for this study. Bailey (1947) gave the taxonomic description of 23 species of jasmine as well as the keys for their identifications. Brief descriptions of eight South Indian species of jasmine, most of them being wild, have been given by Raja (1953). The descriptions, medicinal uses and cultivation aspects of 13 species of jasmine are also elaborated in Wealth of India.
Apart from distribution, flowering period, flower colour and odour of 13 species, ornamental value of 18 other species were listed. Wight (1963) gave the taxonomic description of 21 species along with good illustrations of *Jasminum sambac, Jasminum grandiflorum, Jasminum arborescens, Jasminum flexile, Jasminum rigidum, Jasminum pubescens* and *Jasminum auriculatum*.

Description, distribution and medicinal uses of 11 jasmine species along with illustrations of *Jasminum grandiflorum, Jasminum arborescens, Jasminum sambac* and a few others were reported by Kirtikar and Basu (1918).

### 2.3.2. Chromosome number and mode of specification in Jasmine

Taylor (1945) in his work on the Cytotaxonomy and Phylogeny determined the basic chromosome number of this genus as \( X = 13 \). Krishnaswamy and Raman (1948) recorded diploids, triploids and tetraploids in this genus. They reported that ‘Sujimallige’, ‘Iruvantige’ and ‘Yellusuthu-mallige’ were diploids (2n=26) and ‘Dundumallige’ was a triploid (2n=39). In terms of species, *Jasminum flexile, Jasminum auriculatum* and *Jasminum grandiflorum* are diploids (2n=26) and *Jasminum primulinum* is a triploid (2n=39).

Raman (1955) listed the chromosome number of 17 species that had already been determined and reported that both allopolyploidy and autopolyploidy appear to have played a role in evolution of jasmine. In addition to these he also gave the chromosome number of *Jasminum calophyllum* (wild, 2n=26), *Jasminum rigidum* (both wild and cultivated, 2n=26) and *Jasminum flexile* (wild, 2n=26; cultivated, 2n=52). The chromosome number of cultivated form of *Jasminum calophyllum* and *Jasminum pubescens* were found 2n=52 and 39 respectively (Dutt, 1952). Sharma and Sharma (1958) reported the chromosome number of *Jasminum grandiflorum* as 2n=26 and *Jasminum pubescens* as 2n = 39 and *Jasminum calophyllum* as 2n = 52.

of differentiation in corollas of four varieties in *Jasminum sambac* and from this study on cytology, evolved a scheme for the possible course of evolution of different varieties and species of jasmine and discussed the relationship between simple and compound leaves, and the origin in respect of leaves, calyx and corolla lobes.

Sharma and Sharma (1958) stated that the speciation in this genus has been principally affected by the structural alteration of chromosomes and continuous accumulation of these structural changes has been the main reason for the origin of new species, although polyploidy too has assisted to some extent initially. Gupta and Sharma (1972) reported that polyploidy and gene mutation played a major role in the origin of various species in jasmine. They also indicated that there is great scope for evolving new, attractive, fragrant and extended jasmine bloom for a larger part of the year containing more perfume in it.

2.4 Morphological studies of different species and varieties of Jasmine

2.4.1 *Jasminum sambac* (Linn) Ait

Detailed description of four horticultural varieties of *Jasminum sambac*, namely ‘Sujimallige’, ‘Iruvantige’, ‘Yelusuthu mallige’ and ‘Gundumallige’ were given by Krishnaswamy and Raman (1948). Later, Raman (1955) grouped these varieties based on the shape of leaf and corolla lobes into two groups:


b. Ovate Leaves; Globose Buds– ‘Yelusuthu mallige’ and ‘Gundumallige’

In another study, Raja (1953) indicated *Jasminum sambac* variety ‘Yelu-suthu mallige’ as *Jasminum sambac* variety ‘trifoliatum’ based on the leaves occurring in three’s at the extremity of flowering branches.

Bhatnagar (1956) recorded four distinct sub-varieties in *Jasminum sambac* on the basis of shapes of the flower buds, petals and number of whorls namely,

i.) ‘Motia Bela’ - with double whorl of rounded petals and globular buds

ii.) ‘Bela’ - with double whorl of elongated petals and elliptic buds
iii.) ‘Bela-Hazara Bela’ - with single whorl of petals and elliptic buds
iv.) ‘Mungra’ - with multi-whorls of petals and round bud

Comparisons were also made based on blooming period, time taken by the flower bud to become a flower, weight and diameter of the flower.

Raman et al. (1969) tabulated data on the weight of 100 buds and 100 opened flowers and the season of flowering in 17 varieties of *Jasminum sambac*. The maximum loss in weight was observed in *Jasminum sambac* varieties ‘Double Mogra’ and ‘Big double’, where as minimum was observed in ‘Oosimalli’. In general, the loss in weight was more in the *Jasminum sambac* group compared to other groups. The per cent loss in weight ranged from 3 to 39 in the 17 varieties of *Jasminum sambac*. The shortest duration of flowering was recorded in varieties ‘Big double’ and ‘Small double’ and the remaining varieties had their flowering period from February to October. Based on yield, the varieties were grouped into high yielders - ‘Iruvatchi’ and ‘Gundumalli’; Medium yielders - ‘Sujimalli’ and Khoya’; and low yielders - ‘Maturia’, ‘Ramabanam’, ‘Single Mogra’ and ‘Madanban’.

A total of 15 accessions were studied in *Jasminum sambac* for variability traits, with particular reference to distinguishing characters of economic value of the flower trade. There were marked differences among them in respect of 1) length of pedicel, 2) length of corolla tube and 3) size and shape of flower bud, which are important characters of economic value in flower trade. A quantitative evaluation of important attributes like shape of flower bud, pedicel length, length of corolla tube, keeping quality of the flowers, time taken for the buds to open out fully and fragrance was made. The cultivar ‘Madanban’ ranked first followed by ‘Gundumalli’ and ‘Ramabanam’. It was suggested that spontaneous mutation, natural crossing and simple autopolyploidy may be the the possible causes for the origin of varieties of *Jasminum sambac* (Khan et al., 1970).

2.4.2. *Jasminum multiflorum* (Burm. F.)

Bhatnagar (1960) in his studies on the biology of *Jasminum multiflorum* reported that it is a composite species and has at least four varieties. One variety is the normal ‘Kund’ with oval unpointed petals and moderate size white flowers. The
second ‘Gaint’ is more florigerous with larger pendulous panicles with elongated petals and large size flowers. The third ‘Violet’ resembled ‘Kund’ except for the pointed petals and pink mauve colouration. The petals of the fourth variety ‘Violet’ (five sepals) had flowers smaller to those of ‘Kund’ and had oval shaped petals with pink mauve colouration outside. The sepals of this variety were very small and had very little pubescence unlike the other varieties. These varieties were also evaluated for other characters like blooming period, weight of the flower, length of calyx tube and corolla tube, time taken for full development of flower buds, stigma respectively, pollen viability etc.

Raman et al. (1969) found that this species produced flowers throughout the year with a peak period being May to January. Jasminum pubescens remained fresh for a longer period of time and loss in weight was reported to be 11 per cent.

### 2.4.3 Jasminum communis

*Jasminum communis*, a clone of *Jasminum nitidum* (Syn.), was identified to be a triploid (Khan et al., 1969). This species was reported as a non fruit bearing type and the flowering period extended up to nine months.

### 2.4.4 Jasminum arborescens (Roxb).

Bhatnagar (1960) revealed that the percentage of fruit set was extremely low in this species. According to the findings of Raman et al. (1969), this species had shortest period of peak flowering (March to June) and the per cent loss in weight was six which was comparatively less.

### 2.4.5. Jasminum rigidum (Zenker)

Raman (1955) observed differences in morphological characters in wild and cultivated forms of *Jasminum rigidum*. Wild type had elliptic to ovate to lanceolate leaves (6.0 x 2.5 cm) and corolla lobes (1.5 x 0.5cm). Cultivated type had cordate to ovate to acuminate leaves (7.5 to 8.0 x 6.0cm) and corolla lobes (2.0 x 0.5 cm).

Anonymous (1959) reported that the flowers of *Jasminum rigidum* were white and very fragrant, the season of flowering being short *i.e.*, March to April, but the
experimental findings by Raman et al. (1969) showed that this species flowered in all the twelve months of the year and the peak flowering was observed from July to November. The loss in weight reported by them was ten per cent.

2.4.6 *Jasminum flexile* (Vahl.)

Wild and cultivated forms of *Jasminum flexile* were evaluated for different characters like leaf size and corolla lobe size by Raman (1955). The terminal and the lateral leaf size in wild type was 10 x 6 cm and 9 x 5 cm respectively, whereas in the cultivated type the terminal and the lateral leaf measured 5.5 x 2.5 cm and 4 x 2 cm respectively. The corolla lobes measured 1.5 x 0.7 cm in wild and 1.8 x 0.6 cm in cultivated forms.

Moderately high percentage of fruit set was noticed in this species by Bhatnagar (1960). A study on the flowering season by Raman et al. (1969) indicated that this species flowered throughout the year with a peak period of eight months (April to November). The loss in weight was very less (5%) in this species.

2.4.7 *Jasminum calophyllum* (Wall.)

Raman et al. (1969) stated that this species produced flowers throughout the year with a peak flowering duration ranging from July to November. Flower bud size was small and flowers were found to have less keeping quality and the loss in weight recorded by them was nine per cent.

2.4.8 *Jasminum auriculatum* (Vahl.)

Differences were recorded in plant morphological characters (size of leaf and corolla lobes) in wild and cultivated forms (Raman, 1955) and they are as follows

<table>
<thead>
<tr>
<th>Morphological character</th>
<th>Wild (cm)</th>
<th>Cultivated (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal leaf</td>
<td>3.0 x 2.0</td>
<td>7.0 x 3.5</td>
</tr>
<tr>
<td>Lateral leaf</td>
<td>1.0 x 0.5</td>
<td>2.0 x 1.0</td>
</tr>
<tr>
<td>Corolla lobe</td>
<td>0.9 x 0.2</td>
<td>1.3 x 0.4</td>
</tr>
</tbody>
</table>
Bhatnagar (1960) reported that the percentage of fruit set was quite high in *Jasminum auriculatum*. The loss in weight while opening was less in *Jasminum auriculatum* varieties as compared to *Jasminum sambac* varieties. The per cent loss in weight ranged from 4 to 9 in the five varieties of *Jasminum auriculatum*. Flowering was observed in this species from February to December with a peak blooming during April to August (Raman et al., 1969).

Five morphological variants were spotted in this species and these differed in important floral characters like diameter of open flower, weight of flower bud, corolla tube length, fruits set and style length. The occurrence of such variants has been reported to be either through bud sports or chance hybridization and this offered scope for improvement of cultivated types through hybridization and mutation (Khan and Muthuswamy, 1969).

### 2.4.9. *Jasminum mesni* (Hance.)

*Jasminum mesni* is known to produce flowers only during the month of March (Sudell, 1932). With regard to fruit set behaviour, Bhatnagar (1960) reported very low (18 %) fruit setting.

### 2.4.10. *Jasminum grandiflorum* (Linn.)

Khan *et al.* (1969) analysed the comparative account of morphological characteristics of triploid and diploid forms of *Jasminum grandiflorum*. The triploid was found to be more vigorous with larger leaves and petals, longer peduncles and corolla tube. Fruit set was noticed only in the diploid.

The observations recorded on the per cent loss in weight showed no major difference between the diploid and triploid forms, though the size of the buds in triploid was more than double compared to diploid clone (Raman *et al.*, 1969). They also found that this species flowered in all the 12 months of the year with peak flowering during July to January.

Veluswamy *et al.* (1973) reported experimental findings of varietal trials of *Jasminum grandiflorum*. The study involved six types (five pink and one white), of which one was triploid with pink flowers and the rest were diploids. Considerable
variations were noted in floral characters. Two promising clones were isolated. Fruit set was noticed in all the clones including the triploid in contrast with the findings of Khan et al. (1969) who noticed fruit set only in diploids, while Murthy and Khanna (1971) did not observe fruit set in diploids or triploids.

2.5. Morphological markers

Traditionally, cultivars have been identified by morphological, physiological or horticultural descriptions. These descriptions are largely subject to environmental conditions and human judgment. Development of new cultivars that differ little from the existing cultivars also required more reliable methods to discriminate between plant cultivars. Morphological traits are the oldest and most widely used genetic markers and they may still be considered as optimal for certain germplasm and cultivar management applications, where the cultivars have been classified on the basis of leaf, panicle, fruit and other physical characteristics. However, these characters may change with environmental conditions. Furthermore, the actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have various names (Lakshminarayana, 1980). The prime advantages of the morphological markers are simplicity, inexpensive assays, even herbarium specimens and other dead tissues having effective means, by which cultivars could be identified and verified.

Continuous usage of morphological traits to describe cultivars indicated that these traits remain as descriptors. However, their ability to provide reliable discriminating identification is cumbersome (Patterson and Weatherup, 1984). Increased number of genetically related genotypes within a species or between species released by plant breeders has made identification more difficult. Morphological traits reflect not only the genetic constitution of the cultivars, but also the interaction of the genotype with the environment (G x E) within which it is expressed. Due to G x E effects, it is clearly inappropriate to compare morphological data for varieties that have been collected across different years and / or locations.

Anuradha and Gowda (2000) estimated the natural variation present in morphological characters like growth, yield and inflorescence characters among 25 different cultivars of gerbera for utilization in breeding programme and observed
differences in genotypes. Morphological (vegetative and floral) characterization based on vegetative and floral attributes of 29 genotypes of African marigold were analysed by Singh and Singh (2006) and noted significant variation among germplasm. They attributed the variations to genetic makeup of the genotypes.

Rahayu et al. (2010) reported morphological variation in 48 Hoya multiflora populations at Pangrango National Park in Indonesia. They found variations with respect to stem, leaf and inflorescences and clustered these genotypes into three groups at 12 percent dissimilarity.

2.6. Biochemical markers

The biochemical markers are in popular usage because the variations for these markers are ubiquitous and the variations can be understood in genetic terms. These characters are in routine and widely accepted as a source of reliable data in evolution, taxonomy and genetics ( Tanksley, 1983).

Proteins are molecules with net electrical charges that are affected by pH. They can be separated by electrophoresis on the basis of their net electrical charge, molecular weight, isoelectric point or combinations of these criteria using multidimensional separations (Bietz, 1986). Enzyme polymorphisms have been used successfully to identify cultivars in various horticultural crops, where analysis is carried out based on the proteins extracted to characterize, identify and classify various cultivars of fruits viz. avocado (Goldring et al., 1985), apple (Weeden and Lamb, 1985), loquat (Degani and Blumenfeld, 1986), cherimoya (Ellstrand and Lee, 1987), pineapple (De Wald et al., 1988) and mango (Hemanth, 1999). They have also been used to distinguish hybrids from self (Degani and Gazit, 1984; Goldring et al., 1987; Degani et al., 1989) and zygotic from nucellar seedlings in citrus (Torres et al., 1982; Roose and Traught, 1988); vegetables viz. asparagus (Maestri et al., 1991), cucumber (Meglic and Staub, 1996) and amaranthus (Okeno and Ayiecho, 1996); flowers viz. anthurium (Kobayashi et al., 1987), rose (Kuhns and Fretz, 1978; Milllan et al 1995), camellia (Wendel and Parks, 1983) and carnation (Messeguer and Arus,1985). Isozymes, as genetic markers, have been proven to be reliable, consistent and essentially unaffected by environmental conditions (Torres and Bergh, 1980; Bailey, 1983).
Gupta and Dutta (2005) characterized 30 small flowered chrysanthemum and categorized them based on different morphological and chemical (chlorophyll) characters. They gave important check list information for identification of variety and also selection of desirable characters for inter varietal breeding programme.

Thirteen commercially important cultivars of carnation with different flower colours were selected for assessment of genetic variability using isozymes as markers by Patial (2007) and reported that the polymorphism was found to be 13.8 percent and they grouped into two clusters and three sub-clusters on the basis of genetic distance.

Although isozyme markers provide the basis for relatively simple tool for genetic analysis and linkage studies, it is unlikely that sufficient number of isozymes will be found to saturate the genome completely and uniformly (Tanksley, 1983). The enzymes extracted and subjected to electrophoreeses are a tiny and probably non representative sample of the total array of proteins present in them. Further, isozymes are influenced by stages of development and tissue used for extraction (Feret and Bergmann, 1976).

Even though proteins are products of primary transcripts of DNA, environmental factors can affect qualitative and quantitative levels of proteins and they interact with other compounds. This interaction can detract from the reproducibility of proteins profiles (Higgins, 1984). The other disadvantages like availability of limited number of enzymes loci, developmental and seasonal dependant expression. In this regard use of reliable gene markers is of great value.

2.6.1. Molecular markers

Among several of the recently developed DNA-based markers, Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Randomly Amplified Polymorphic DNA (RAPD) markers are excellent tools to study the genetic diversity, eliminate duplicates in germplasm, study genetic relationships, gene tagging, genome mapping, PVR (Plant Varietal Rights) purposes etc. These markers measure diversity at DNA level and are seldom influenced by environmental conditions.
2.6.2. DNA Isolation Protocols

The DNA content in higher plants is highly variable. Armuganathan and Earle (1991) estimated the DNA content in over 100 important crop species. DNA content varied from 0.30 picogram (pg) per 2C nuclei or 145 million base pairs (mbp) in *Arabidopsis* to over 50 pg or 24,255 mbp in leek. However, the DNA content of most of the intensively mapped diploid species (tomato, rice *arabidopsis*) was in the range of 0.30 to 1.0 pg. *Fraxinus americana* L. (diploid), which belongs to the same family of jasmine (‘Oleaceae’), recorded a DNA content of 3.2 pg per cell (Bennet *et al.*, 1982).

Murray and Thompson (1980) tried to isolate high molecular weight DNA that was free of contaminants. In this, the dry powder was mixed with extraction buffer (0.7 M NaCl, 1 % CTAB, 50 mM Tris HCl with pH 8.0, 10 mM EDTA and 1% - Mercaptoethanol) at the rate of 1 ml per 30-100 mg of powdered sample. The mixture was incubated for 20-30 minutes at 50-60 °C with occasional gentle mixing. The extract was emulsified with an equal volume of chloroform / Octanol (24:1) and centrifuged. To the aqueous phase one –tenth volume of 10 per cent CTAB, 0.7 M NaCl added and the chloroform / octanol treatment was repeated. After 30 min at room temperature, the precipitate was recovered by low speed centrifugation. The CTAB nucleic acid precipitation was dissolved in 2ml of buffer solution containing 1M CsCl, 50 mM Tris HCl, 10mM EDTA and 200 µg per ml ethidium bromide. The solution was placed in the ultra centrifuge tube and 2.4 ml of another buffer solution containing 6.6 M CsCl, 50 mM Tris HCl (pH 8.0), 10 mM EDTA and 1 per cent sarkosyl were added allowing considerable mixing to perform a crude density gradient. The DNA was visualized under long wave UV illumination.

According to Richards (1987), the DNA isolation procedure involved cell lyses by detergent, protease treatment and CsCl gradient purification. Due to total cell lysis the DNA purified using this protocol will correspond to both the nuclear genome and cytoplasmic genome (mitochondrial and chloroplast). The methods described by Watson and Thompson (1988) was followed for purifying nuclear DNA free of plastid and mitochondrial DNA contamination. A mini preparation protocol developed by
Delloporta et al. (1983) was used for isolation of DNA omitting the CsCl gradient purification.

Tai and Tanksley (1990) described an inexpensive method for dehydration of plant tissue and extraction of high molecular weight DNA. Tissue was dried for 12 to 24 hrs in a food dehydrator and subsequently powdered for DNA extraction. Dicot tissue can be powdered in centrifuge tubes using a commercial paint mixer. Tissue never touches the common surfaces that might lead to cross contamination- a potential benefit when the DNA is to be used for PCR reactions. The DNA is of quality, equal to that obtained from either lyophilised or fresh or frozen tissue. The advantages of the described procedure are that it is fast, does not require expensive equipment (e.g. lyophilizer) and can be used in situations where large number of samples have to be extracted.

A rapid, simple and efficient protocol was given by Alexander and Serge (1990) for the extraction of restrictable total DNA from plants of the genus *Abelmoschas*, for which the main obstacle was stickiness of the solution after grinding the green leaves. This problem was solved using cotyledons of dark grown seedlings. The DNA isolation purification process modified from Delloporta et al. (1983) yielded about 100 µg of total DNA from one gram of cotyledons from several samples and does not require the use of sophisticated equipment. The DNA obtained was clean and restricted by all the restriction enzymes.

A method described by Varadarajan and Prakash (1991) is capable of extracting high quality DNA from plant species having interferences from the secondary metabolites, latex/ mucilaginous secretions, polysaccharides and protein. Little modifications from Delloporta et al. (1983) protocol was found successful in DNA extraction with diverse plants such as *Petunia*, *Glycine* and *Nicotiana*. The procedure had three main departures from Delloporta et al. (1983). Pre-treatment of tissue prior to cell disruption, recovery of DNA from the insoluble gelatinous mass by processing this as the second fraction and additional periods of centrifugation and ethanol washes of DNA pellets helped in purification. Concentration was made possible with an average recovery up to 150 µg of DNA per g of leaf tissue from gelatinous suspension.
A quick and inexpensive method was demonstrated to remove polysaccharide contamination from plant DNA. Isolated plant genomic DNA with polysaccharide contaminants was dissolved in TRIS extractant (10 mM Tris HCl with pH 7.4 and 1mM EDTA) and with varied NaCl solutions (ranging from 0.5 – 3.0 M) and then precipitated with two volumes of ethanol. Most of the polysaccharides were removed effectively in a single high salt precipitation at 1.0-2.5 M NaCl. At 3.0 M NaCl the salt precipitated out of solution. Purified DNA was easily digested by either Hind III or Eco RI and was found satisfactory as template for PCR. The results showed that high salt precipitate effectively removed polysaccharides and their inhibitory effects on restriction enzymes and Taq polymerase activity (Fang et al., 1992).

According to Steiner and Hoff (1995) an ideal technique for DNA extraction should minimize the number of times a tissue sample is handled from collection to analysis, optimize yield of DNA extracted from a sample, be suited to mass handling of samples minimizing labour and material costs and does not generate hazardous waste that may negatively impact the environment. They demonstrated a novel DNA extraction process performed in a single tube from the time a tissue is collected until diluted aliquots of the extract are taken for PCR reactions. The process did not require centrifugation, can prepare up to 6000 samples per day, resulted in enough DNA extraction to perform 4000 PCR amplifications per sample and could be used for plant, animal and microbial source of DNA.

Porebski et al. (1997) described a relatively quick, inexpensive and consistent protocol for extraction of DNA from expanded leaf material containing large quantities of polyphenols, tannins and polysaccharides. Matured strawberry leaves, containing high levels of the secondary components, were used as a study group. The method involved a modified CTAB extraction, employing high salt concentrations to remove polysaccharides, Poly Vinyl Pyrrolidone (PVP) to remove phenols, an extended RNAse treatment and phenol-chloroform extraction. Average yields ranged from 20-84 µg per g, mature leaf tissue for both wild and cultivated octoploid and diploid Fragaria sp. Results from 60 plants were examined and were consistently amplifiable in the RAPD reaction with as little as 0.5 ng DNA per 25 µl reaction. Presently, this is the first procedure for the isolation of DNA from mature strawberry leaf tissue that produces consistent results for a variety of different species, both
octoploid and diploid, and is both stable and PCR amplifiable before and after extended storage.

According to Boilteux et al. (1999) seven plant genomic DNA purification protocols were evaluated for generic fingerprinting analysis using six tissues obtained from inbred carrot (*Daucus carota* L.) lines. Evaluations were made based on the yield, purity, cleavage with Hind III, integrity and suitability for amplification in RAPD system. Significant differences were observed among tissues and purification methods for the total amount of DNA. An extraction method using CTAB buffer and organic solvents gave the best results in DNA yield, purity and Hind III cleavage when compared with other six non-organic extraction methods. Of the tissues examined, flowers yielded the most DNA (average value = 115 ng of DNA per mg of fresh tissue) followed by seeds (54 µg per mg), fresh leaves (48 ng per mg), lyophilized leaves (40 ng per mg), calli (22ng per mg) and tap roots (4 ng per mg) for most of the preparations, the DNA showed no traces of degradation. However, DNA preparations were not consistently accessible to Hind III cleavage in all tissue extraction method combinations. Uncut DNA was observed chiefly in extractions from flowers and fresh leaves suggesting a tissue specific adverse effect on restriction endonuclease activity. Differences in RAPD band intensity and number were observed across tissues and DNA extraction methods using identical PCR conditions for RAPD. Callus was the best type of tissue for RAPD based fingerprinting yielding a consistently higher number of more intense amplicons when compared to other tissues. In flowers and seeds, the DNA obtained by using the CTAB extraction method was amplified. Polymorphism deviating from generic expectations were mainly observed in root and fresh leaf DNA, indicating the same RAPD markers may not present satisfactory levels of reproducibility. Judicious and uniform selection of DNA purification method as well as tissue for DNA extraction is therefore, important considerations for reliable RAPD based DNA finger printing. In addition, the studies allowed identification of better combination of procedures for use in routing manipulations of DNA such as RFLP – RAPD based cultivar finger printing, molecular mapping, screening of transgenic plants, construction of genomic libraries and gene cloning.
Lim et al. (1998) reported a simple and reliable method for extracting quality and amplifiable DNA in orchid species and hybrids. Puchooa and Khoyratty (2004) have developed the first reliable and efficient method for isolating *Victoria amazonica* genomic DNA that is free from polysaccharides and polyphenols. They used extractant solution containing 1.5M NaCl, PVP (2 %), mercaptoethonal (5 %) and sodium sulphate (0.12 %) and incubated at 65 °C for 4 hours. They obtained 387 µg per g of leaf material and it proved amenable to restriction digestion.

A method for extraction of high quality DNA from four *Opuntia* sp and other *Cacti* using CTAB is described by Jacobo et al. (2000). These plants typically contain high levels of mucilages and complex polysaccharide compounds restricting pure DNA extractions. The method involves adjusting the amount of tissue used according to species and age, followed by processing in an extraction buffer to separate coarse material. Extended centrifugation and digestion time in a separation buffer with CTAB (2 %) was adopted. Exposing tissue to both buffers maintained polysaccharides in solution and allowed easy recovery of the aqueous phase containing DNA. 5-8 g tender tissue was needed to obtain 153 µg DNA and old tissue yielded (26 %) less DNA. Extraction of DNA from 5g samples of tender tissue of the ornamental cacti *Stenocereus* sp., *Cleistocactus* sp. and *Echinocereus* sp. was successful. For these species, average yields ranged from 25 to 53 µg per sample. The DNA obtained was suitable for PCR amplification, producing clear, distinctive and reproducible banding patterns useful for a variety of applications.

Most higher plants have a considerable portion of DNA as repetitive non coding DNA that is not transcribed. Species with large genomes have more repeated DNA and higher proportion of repeated DNA to single copy DNA ( Tanksley and Pichersky, 1988). Thus, only small fraction of the total genetic variation at a DNA nucleotide sequence level reveals itself as a distinct trait, showing Mendelian inheritance. The degeneracy of the genetic code ensures that one in three nucleotide changes will not affect the amino acid sequence of protein produced. Thus, the great bulk of genetic variation at the nucleotide level may not have any detectable expression at phenotypic level. It is this genetic variation that is expressed as DNA based polymorphism.
2.6.3. Restriction Fragment Length Polymorphism (RFLP)

This method involves digestion of the genomic DNA with cleaving restriction endonucleases (Grodzicker et al., 1974), fractionating the fragments electrophoretically and then preferentially visualizing fragments containing particular homologous sequences by hybridizing them to specific DNA probe. RFLP analysis has been used to fingerprint ornamentals such as hybrid teas and miniature roses (Hubbard et al., 1992; Rajapakse et al., 1992), dogwood (Culpepper et al., 1991), chrysanthemum (Wolff et al., 1993), china aster (Suneetha, 2000), anthurium (Basavarajappa, 2000), Jasmine (Mukundan, 2000), garden petunias (Zhang et al., 2008). It is also used to construct the linkage maps for many important crops such as lettuce (Landry et al., 1985), maize (Helentjaries et al., 1986), Arabidopsis (Chang et al., 1988), pepper (Tanksley et al., 1988), rice (Mc Couch et al., 1988), tomato (Paterson et al., 1988; Tanksley et al., 1988), potato (Gebhardt et al., 1989), lentil (Havey and Muehlbauer, 1989), Brassica sp. (Slocum et al., 1990; Landry and Hubert, 1991; Song et al., 1991), barley (Huen et al., 1991) and sorghum (Whitkus et al., 1992). However, RFLP analysis is relatively labour-intensive, expensive and involves use of radioactive chemicals, which are harmful to the users.

2.6.4. Amplified Fragment Length Polymorphism (AFLP)

The technique involves restriction of the DNA and ligation of oligonucleotide adapters, selective amplification of sets of restriction fragments and gel analysis of the amplified fragments. AFLP technique is a very effective tool to reveal restriction fragment polymorphisms. It can generate fingerprints of any DNA regardless of the origin or complexity and construct genetic marker maps (Vos et al., 1995). This technique has been demonstrated in tea (Paul et al., 1997), neem (Singh et al., 1999) and eucalyptus (Marques et al., 1999). However, AFLP as a technology is intensive and involves use of radioactive probes.

2.6.5. Randomly amplified polymorphic DNA (RAPD) markers

The discovery of Polymerase Chain Reaction (PCR) has led to the development of another genetic marker system for detecting polymorphism called Randomly Amplified Polymorphic DNA (RAPD) (Mullis, et al 1986; Mullis and
Faloona, 1987; Welsh and Mc Clelland, 1990; Williams et al., 1990). This procedure detects nucleotide sequence polymorphisms in a DNA amplification based assay using only a single primer of arbitrary sequence using Polymerase Chain Reaction (PCR).

In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these sites are within amplifiable distance a fragment is amplified. The presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and the oligonucleotide primer at each end of the amplified product. On an average, each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals. The major advantage of this assay over RFLP method is that there is no prior requirement for DNA sequence information of the genome. The protocol is also relatively quick and easy to perform and uses fluorescence in lieu of radioactivity (Tingey and Del-Tufo, 1993). The RAPD technique is amplification based assay, automation is feasible and only nanogram quantities of DNA are required for analysis.

The RAPD protocol is simple and fast. The procedure involves extraction of DNA from different accessions, by any of the standard protocol and amplifying the DNA by PCR using random primers. The profiles of accessions are scored for presence or absence of bands directly by running agarose gel, stained with ethidium bromide. Since RAPD approach is being adopted in the present study much of the literature reviewed pertains to use of this technique.

2.6.6. RAPD markers in ornamentals crops

RAPD markers have also been used extensively in ornamental crops for discriminating cultivars, genetic purity, genetic diversity, phylogenetic relationship and crop improvement programmes in ornamental crops.

Matsumoto and Fukui (1996) used RAPD markers successfully for the identification of nine rose cultivars and three clonal plants. All the cultivars were identified by using only three primers. Unique RAPD marker bands were used in
distinguishing individual cultivars. Gallego and Martinez (1996) also used molecular typing of 25 rose cultivars using twenty 10-mer primers. The data obtained revealed no variability within cultivars and a high degree of variation between cultivars. Based on the patterns obtained with two of the primers, all the rose cultivars were unequivocally identified.

One hundred nineteen accessions belonging to the genus Rosa and 213 markers of 36 rose species that include 8 sections of sub-genus Eurosa and one species each from the subgenera Hesperhodes and Platyrhodon were used to calculate a similarity matrix, which was clustered with the Unweighted Pair Group Method Analysis (UPGMA) using arithmetic means. The RAPD markers distinguished between all the rose accessions and species grouped in to their respective sections. Classification of Rosa using RAPD data supported traditional classification (Jan et al.1999).

Genetic variation in chrysanthemum was studied using RAPD technology by Wolff et al. (1993). Variation between cultivars was high and cultivars used could be distinguished from each other by using only two different primers. A family of cultivars, derived from one original cultivar by vegetative propagation, had identical fragment patterns. Because of high level of polymorphism and clonal stability. RAPD fragments were useful for cultivar identification. Scott et al. (1996) also reported that 22 cultivars of chrysanthemum belonged to ‘Anne’, ‘Blue’, ‘Boaldi’, ‘Charm’, ‘Davis’ and ‘Pomona’ series. The genetic variability of cultivars within and between series was also evaluated.

Iqbal et al. (1995) showed that RAPD markers have the potential to reveal genetic similarity among plants. Profiles of thirteen Rhododendron species, hybrids and cultivars were analyzed to study their genetic relationships. The cluster analysis grouped together varieties and/or hybrids in accordance with their known genetic relationship. For instance, two varieties of Rhododendron yakushimanum, var. ‘Mist Maiden’ and var. ‘Ken Janeck’, clustered together while Rhododendron yakushimanum var. ‘Pink Parasol’ clustered with a hybrid of ‘Pink Parasol’ x Rhododendron smirnowii. These three varieties and the hybrid then clustered with Rhododendron smirnowii. Similarly, the other hybrids with common parents showed a
closer genetic relatedness with each other than with other *Rhododendron* sp. The genetic relationship revealed from cluster analysis on the basis of RAPD profiles was similar to their known genetic makeup.

Nucleic acid extracts from 23 *Alstroemeria* cultivars were amplified with 8 random decamers by PCR. The distinctive RAPD patterns generated from these cultivars could be used as genomic fingerprints to establish the identity of a given genotype. The ‘Orchid’ and ‘Butterfly’ types were clearly separated in distinct subclusters in a phyllogram obtained by UPGMA genetic distances. The ‘Hybrid’ types were distributed in two major subclusters, reflecting the diversity of the parental species used to generate the population. This phyllogram conformed to expectations based on the available pedigree data (Joseph *et al.*, 1997).

Fingerprinting of 34 *Pelargonium* genotypes was carried out on the basis of their amplification profiles. Multiple band profiles were generated using 23 selected primers, leading to 96 markers (76% of them polymorphic). The results clearly indicate that RAPD markers are suitable for *Pelargonium* varietal identification (Renou *et al.*, 1997). In *Heliconia* species, the identification is done primarily based on morphological differences as they are polymorphic in nature and with a large number of cultivars. The percentage similarity among *Heliconia* species, cultivars and hybrids were determined. Data from 11 primers indicated that RAPD technology is useful in distinguishing species and cultivars of *Heliconia*. Using a single 10-mer primer distinct RAPD profiles for 16 cultivars of *Heliconia psittacorum* could be generated. The triploid cultivars of *Heliconia psittacorum* showed identical RAPD profiles with 10 different primers, suggesting that they are of same genotype (Prakash *et al.*, 2005).

RAPD markers were successfully used to generate a RFLP marker, which could discriminate with 100% accuracy, between semi-double and double-flower phenotype in carnations of both Mediterranean and American groups. RAPD marker co-segregating as homozygous for recessive allele was cloned and used as RFLP probe for further use in discrimination (Scovel *et al.*, 1998).

Lim *et al.* (1999) reported on the genetic closeness of various species of Vanda using RAPD markers. Strap leaved *Vanda* species (including *Vanda*
sanderiana) and Asecentura miniatum were more closely related to each other than
to the terete-leaved Vanda species studied. RAPD analysis supported the suggestions
that terete-leaved Vanda terete and Vanda sanhioriana should remain in the genus
Vanda. Likewise, Starman et al. (1999) investigated the DNA amplification
fingerprinting to evaluate the genetic relationship among 11 cultivars of Poinsettia.
Thirty one per cent of the bands were polymorphic and distinguished among cultivars.

2.7. Application of Markers

2.7.1. Discriminating Cultivars

Torres et al. (1993) have used RAPD markers to identify Rose cultivars like
primers. They reported that the differences among cultivars were obvious and
expressed constantly with most primers. The most discriminatory primers were OPA-
05, OPA-06 and OPA-08, which gave clear differences in banding patterns among
cultivars. Using OPA-06, ‘Sonia’ was discriminated from all other cultivars by the
simultaneous presence of two intense bans 950 bp and 1100 bp, while ‘Carta Blanca’
exhibited the presence of 950 and 800 bp bands. Further, they have reported that this
method is rapid and relatively inexpensive way to resolve many highly discriminatory
bands. Thus, this technique increases the probability of identifying the cultivars
correctly to protect patent rights.

According to Lin-Wu and Hong-Lin (1994), the PCR and RAPD were
potentially useful methods for identifying turf grass breeding lines. RAPD markers
were studied in 25 vegetatively propagated buffalograss lines using oligonucleotide
random primers and agarose gel-electrophoresis to determine their potential for
identifying breeding lines. The variations of RAPD markers were extensive. The
RAPD marker produced by one random primer was sufficient to discriminate 25
buffalograss lines. Cluster analysis based on RAPD markers produced by two random
primers revealed that 25 buffalograss lines generally fall into two groups: diploid and
haploids.

Huff et al. (1999) described that the population genetic variation of RAPD
markers in natural diploid sources of dioecious buffalograss. Most of the sources of
buffalograss were polyploid and diploid population, which were previously known only from semi arid central Mexico. According to the new Analysis of Molecular Variance (AMOVA) technique among individuals within and among populations; within and among adaptive regions, there was a considerable variation within each of the four populations. The largest observed genetic differences were those between two regional ecotypes.

Yamagishi (1995) used RAPD markers for the identification of *Lilium* species and interspecific hybrids. The optimum annealing temperature of PCR reaction for the RAPD array in *Lilium* was 54 °C, which is relatively higher. Among 76 primers used, 18 primers (24 %) generated polymorphic DNA fragments in *Lilium* species and hybrids. RAPD markers also identified cultivars. Some amplified fragments were unique to species of each section and to the hybrids derived from these species. Sections of Sinomartagon, Leucolirion b, Leucolirion and Archelirion could be identified by section specific markers amplified with five primers. Seven intersection hybrids showed the section-specific bands of both parental sections, indicating that these markers could be useful for identifying the parental section of inter-section hybrids.

Sweeny and Danneberger (1995) used eight primers to screen the differences between bulk samples of greens and fairways of *Poa annua*. Nine primers produced 12 RAPD markers. Five of these were used to evaluate 54 individual plant samples from each environment. Statistical evaluation indicated significant differences (P=0.01) between the green and fairway populations for four of the RAPD markers and among holes within fairways for these markers. These differences have indicated a limited gene flow between adjacent populations of *Poa annua*.

Anastassopoulos and Keil (1996) used RAPD markers to study genetic variation in *Alstroemaria*. All the genotypes were examined, including commercial varieties. Out of 13 mutants, eight *Alstroemaria* plants obtained after irradiation or tissue culture did not show polymorphisms when compared to control plants that were considered to be non-mutated. Only five of the mutant plants analysed expressed polymorphisms. These results suggest that frequent genome rearrangements had not occurred in the mutant plants analysed and it is clear that RAPD technique is an
appropriate tool for the rapid screening of *Alstroemaria* for induced variation. It seems probable that these conclusions could be equally applicable in other plant general in which induced variation has occurred. However, the RAPD technique is a simple and effective tool for genetic finger printing of *Alstroemaria* varieties provided their differences are due to sexual propagation.

Millan *et al.* (1996) analysed nineteen species of Rose using RAPD markers. Each 10 base long arbitrary primer produced a specific banding pattern that grouped plants belonging to the same species and botanical sections as predicted from their genetic background. Some 175 amplification products were examined by cluster analysis to assess their genetic relationships among species and their genetic distances. All the accessions belong to one species grouped together before branching to other species. Dendrograms constructed for intra and inter-specific studies showed a good correlation with previous classification by different authors based on morphological and karyological studies. These results showed that the RAPD technique is a sensitive and precise tool for genomic analysis in rose, being useful in assigning unclassified accessions to specific taxonomic groups or else following accessions classified by traditional criteria to be re-classified.

Out of 90 primers tested in poppies, 38 markers revealed inter-species genetic polymorphism between individuals of the two species from geographically isolated population. However, intra-species homogeneity was also evident with respect to a number of primers both within and between populations. The overall representation of relationships by cluster analysis was similar for all the three marker (RAPD, finger printing and isozymes) systems. This was substantiated by high correlations among these analysis revealed by the Mantel-Mantrix correspondence test. These results point to very low or absence of genetic polymorphism in *Meconopsis paniculata* and *Meconopsis simplifolia*. They are in broad agreement with previous observations on genetic diversity of *Meconopsis* species which point to a genetic basis for the possible extinction of this economically important genus (Sulaiman and Hasnain, 1996).

Ling *et al.* (1997) compared the DNA from leaf tissues of nine commercial *Poinsettia* cultivars. Amplification occurred in 57 out of 60 (95 %) tested primers. Nine primers that revealed polymorphism among cultivars were selected for further
evaluation. Forty eight RAPD bands were scored from three primers and 33 (69%) were polymorphic. All the tested cultivars could be discriminated with seven bands generated from primer OPB-7 and OPC-13. Results of UPGMA cluster analysis and PCA placed the nine cultivars into two groups. One group consisted of ‘Jingle Bells’, ‘Supjibi’ and ‘V-17 Angelika’, the other of ‘V-14 Glory’, ‘Red Sails’, ‘Olly Red’ and ‘Freedom’. ‘Lelo Red’ and ‘Pink peppermint’ belonging to the latter group, but were relatively distant from other cultivars in that group. These results indicated that RAPDs were efficient for identification for Poinsettia cultivars and for determination of the genetic relationships among cultivars.

Gehring et al. (1997) showed a number of PCR amplification products, which varied from 8 -16 per cent in Kalanchoe. The UPGMA and the neighbor joining methods have used for construction of phylogenetic trees. Irrespective of the mathematical method used for the construction of the dendrograms on the basis of amplification product pattern they investigated species clearly grouped into three sections of the genus and thus, with the infra generic distribution of the three types of CAM performers.

The generic relationships of Viola sp were analysed using the RAPD method by Ko et al., (1998). They also compared clustered groups from RAPD analysis with the conventional taxonomy based on morphological characters. Forty random primers were selected for PCR analysis. UPGMA analysis clustered the Viola spp. into two groups A and B. The generated data was consistent with conventional taxonomy based on morphological characters, such as leaf form, vein patterns and stigma shapes. Group A was divided into three subgroups namely AI, AII and AIII. This study demonstrated that the genetic relationships of Viola species derived from RAPD analysis was in agreement with conventional taxonomy based on morphological characters except the subgroup III. RAPD analysis was applied to deduce putative parents for six unknown hybrid lines with their closely related Viola spp. A total of 44 different amplification fragments by the selected primers scored 82 per cent showed polymorphism between samples.

RAPD data from 11 primers in Heliconia species, cultivars and hybrids suggested that 10 mer primer (OPA 18) could produce distinct RAPD profiles for 16
cultivars of *Heliconia psittacorum*. The phylogenetic tree derived from RAPD data showed that all the 16 cultivars examined are closely related to each other, providing the first genetic evidence that this large group of cultivars had a common background. Two triploid cultivars of *Heliconia psittacorum* (Iris and Petra) showed identical RAPD profiles with 10 different primers in agarose and polyacrylamide gel suggesting that they are of the same genotype (Kumar *et al.*, 1998).

A total of 132 RAPD markers were produced from fifteen, 10-mer arbitrary primers in *Cymbidium* of which 78 per cent were polymorphic. All cultivars were distinguishable when a number of primers were considered. ‘Blue Smoke’ and ‘Green Meadow’ were distinguished from all others based only on the lack of the OPA5-370 fragment. Genetic distances among the cultivars were estimated based on the amount of band sharing and ranged from 0.08 to 0.50, with an average of 0.29. Cluster analysis of genetic distance estimates grouped siblings and parents with offspring, thereby agreeing with known parentage information and substantiates isozyme data obtained from a separate study (Okeyo and Kako, 1998).

Jinhua *et al.* (1997) showed that the use of RAPD markers as a means to identify genetic purity in six cultivars of *Petunia* seedlings and five cultivars of *Cyclamen* seeds. Evaluation of individual seeds from a single cyclamen hybrid produced polymorphic banding patterns that were attributed to genetic variability present in the female and male inbred parents.

Alice *et al.* (1999) demonstrated that *Juniperus sinensis* and *Juniperus sabina* are closely related using PCA of 122 RAPD bands. Cultivars of Pfitzer groups lacked affinity with either species, but stood apart as a cluster. The morphological data supported that the pfitzer group was separate from *Juniperus chinensis* and indicated the hybrid origin from parents *Juniperus chinensis* and *Juniperus sabina*. Finally, they recognized Pfitzeriana (spath) scalamidt (pfitzer group) as the correct name of cultivars *Pfitzer juniperus*.

2.7.2. Determining genetic purity

Walker and Werner (1997) attempted to analyze the ‘Cherokee’ rose for its putative hybrids ‘Silver Moon’ and ‘Anemone’. RAPD analysis carried out with
sixteen primers revealed forty reproducible polymorphisms. Based on the number of RAPD bands resolved in ‘Anemone’ (98) and ‘Silver Moon’ (90), their respective similarities to ‘Cherokee’ rose were 69.4% and 22% suggesting ‘Cherokee’ rose parentage for ‘Anemone’ but arguing against ‘Silver Moon’.

RAPD markers were used to assess the relationship among species, cultivars and hybrids of lilac. Thirteen random primers were used to examine 87-130 bands per cultivar or hybrid. The percentage band sharing among Syringa x Chinensis cultivars ‘Alba’, ‘Saugaena’ and ‘Red Rothomagensis’ and Syringa x periaca was surprisingly high. It was felt that RAPD markers were very useful for assessing the origin of cultivars and hybrids of many plant species (Marsolais et al., 1993).

Padgett et al. (1998) investigated the plants intermediate in appearance between Nuphar microphylla and Nuphar variegata (Nymphaeaceae) that have long been assumed to be the result of hybridization. They employed multivariate analyses of morphology, pollen fertility studies and RAPD markers to test the hypothesis that Nuphar x Nuphar rubrodiscis represents a natural interspecific hybrid between Nuphar variegata. Examination of 15 morphological characters demonstrated the intermediacy of Nuphar x rubrodiscis between Nuphar microphylla and Nuphar variegata, and the pollen data revealed a markedly lower mean pollen viability in Nuphar x rubrodiscis (23 %) compared to the other two species (91 and 86 % respectively) Eight 10-mer primers produced 13 species specific RAPD markers for Nuphar microphylla and nine for Nuphar variegata, with all 22 markers present in Nuphar x rubrodiscis. The data from RAPDs were concordant with morphology in implicating Nuphar microphylla and Nuphar variegata as parents of Nuphar x rubrodiscis.

2.7.3. Estimating genetic diversity

RAPD-PCR analysis was applied to compare 30 Kalanchoe species with ten 10-mer synthetic oligonucleotides to amplify multiple PCR products with genomic DNA as template. Four primers exhibiting between eight and sixteen distinct fragments, were chosen for data analysis to generate dendrograms. This resulted in nearly identical dendrograms showing that within the genus the species form three
clusters, which coincide well with three intra-generic groups of species distinguishable by taxonomical criteria (Hans et al., 1997).

Wang and Bao (2005) estimated genetic relation between 18 inbred lines of Pancy (Viola vitrockiana) by morphological data and RAPD analysis. The RAPD analysis using 21 primers, produced 127 polymorphic bands from 0.2 kbp to 2.0 kbp. They found several reasons for the differences between the morphological and molecular classification.

In Plantago major, an ornamental cosmopolitan species, RAPD markers were used to study the genetic divergence in two subspecies namely major and pleiosperma. A total of thirty-four bands were scored, of which ten were monomorphic. Substantial polymorphism was revealed of the remaining twenty-four bands using three RAPD primers. Most of the variation found by polymorphism (19 out of 24 bands) was between subspecies (Wolff and Richards, 1998).

RAPD analysis was used effectively as an alternative classification system to identify genotypes and morphological characters (Ko et al., 1998). The genetic relationship of Viola species in the subsection Patellares were analysed using RAPD method. Viola species from the series Chinensis and Pinnatae, and five hybrid lines formed three different clusters indicating subgroups. Viola species in the series Variegatae and a hybrid line were clustered into a second group. The clustered subgroups from RAPD analysis were in agreement with the conventional taxonomy based on morphological characters.

Hong et al. (1996) studied phylogentic relationship among 10 ornamental alliums using RAPD analysis. Wide variations in banding profiles among species were observed with each of the nine-decamer primer tested. Genetic similarities between species studied through dendrogram showed phylogenetic relationships by analysing 265 bands and cluster analysis. The resulting analysis agreed with the previous classification of the species to a certain degree, although it showed a closer relationship between Allium albopilosum [Allium christophii] and Allium giganteum.
Gallego and Martinez (1998) observed variability within rose cultivars and high degree of variation between cultivars when genomic DNA from 25 cultivars of rose were amplified using ten RAPD primers.

Jan et al. (1999) analyzed 119 accessions belonging to genus Rosa and 213 markers of 36 rose species that include eight sections sub genus Eurosa and one species each from the sub genera Hesperhodes and Platyrhodon. The RAPD markers distinguished all the rose accessions and species in accordance with the traditional classification.

Meir and Vainstein (1993) reported that DNA fingerprint analysis using mini-satellite probes (MSP) correctly reflected relationships within and between categories of rose plants. Nearly identical DNA fingerprint patterns were observed for cultivars ‘Dick Koster’ and its two sports. A high level similarity was detected between genotypes of the hybrid tea category while a low level similarity was detected within the miniature category. Highest levels of similarities were detected between hybrid tea and floribunda categories while, the lowest similarities were recorded between hybrid tea and miniature categories. Comparisons of a wild species (Rosa cannina) with all cultivated hybrid categories revealed very large differences.

Arbitrary Primer Polymorphic DNA was employed by Ho et al. (1997) to investigate relationships among 18 Cynodon cultivars. Thirteen out of the 20 primers screened gave reproducible banding patterns for all samples. The cultivars showed a high level of polymorphism. One primer was able to discriminate between all the cultivars except Tif dwarf and it’s off type sample. The Cynodon grasses used in this study separated into two distinct groups based on a distance matrix calculated from the DNA amplification data.

Nearly 26 plant clusters of Sedum integrifolium from four of the five known populations were assayed for the evidence of clonal reproduction using 28 RAPD markers. Of the 81 stems, 75 had unique genotypes and three pairs had identical genotypes suggesting that clonal reproduction is infrequent (Olfelt et al., 1998).

The reliability of RAPD techniques to amplify polymorphisms in Asparagus officinalis was investigated by Hollingsworth et al. (1998). Six cultivars were used in
this study and distinguished by analyzing unique banding patterns of each primer. OPC-12 generated polymorphic markers unique to three of the cultivars investigated. Results indicated that RAPD markers could be used to characterize cultivars were sensitive enough to reveal differences within seed raised commercial cultivars.

Wolff et al. (1993) optimized the generation of RAPDs in chrysanthemum and reported that the polymerase brand, thermal cycler brand, annealing temperature and primer are important factors in obtaining optimal fragment patterns. They suggested that suitable primers for species must be determined by trial and error.

Several techniques of DNA analysis were applied by Wolff et al. (1995) to identify chrysanthemum cultivars. They could distinguish unrelated cultivars by RAPDs and inter SSR, PCR, hybridization based DNA finger printing as well as RFLPs. Cultivars with different flower colours and belonging to one group of vegetatively derived form from one cultivar appeared to have the same DNA fragment patterns whichever technique was applied. The absence of polymorphism between different accessions of the same cultivars indicated a high stability of the observed patterns.

Genetic profiling of six Red bracted Cornus kousa (flowering dogwood–a landscape plant) cultivars was characterized by Trigiano et al. (2004) using DNA AFLP and ASAP. These two methods produced consistent genetic similarities in three red cultivars. The methods were able to identify and separate one red cultivar and two white cultivars.

Sauve and Zhou (2005) used RAPD technique to identify and determine the phylogenetic relationships of 37 Hosta accessions representing the major subgenera, sections and groups. The results of their study showed that RAPD markers differentiated not only the main groups (sharing many genetic traits) but also cultivars within a species.

Gulsen and Shearman (2005) used SRAP markers to estimate diversity and relationships among 56 naturally occurring buffalo grass genotypes collected from diverse geographic locations. All genotypes discriminated from each other with
similarity values ranging from 0.70 to 0.95 PCA suggested that 56 genotypes could be reduced to 50 due to high similarity levels among some of the genotypes.

Kumar et al. (2005) assessed the polymorphism and genetic relatedness among 21 Indian bred rose cultivars based on RAPD markers. The percentage polymorphism ranged from 66 to 100 and the bands were in the range of 0.40 to 2.40 kbp and grouped into four major clusters.

Molecular characterization of four species and 13 varieties of Heliconia using RAPD markers by Sheela et al. (2006) showed the formation of nine distinct clusters of similarity coefficient value of 0.42 among 17 genotypes and as per their expectation taxonomically related genotypes clustered together and the distant ones segregated.

Sasikumar et al. (2007) characterized 25 rose cultivar and 5 species by RAPD markers using 35 random decamer primers. They reported that 28 primers generated polymorphic bands and 25 amplified all the rose cultivars. The cluster analysis grouped 25 cultivars into 3 major clusters and five species formed a separate cluster. They indicated that RAPD technique as an efficient, reliable and clonic alternative to the conventional morphological marker based methods.

Target Region Amplification Polymorphism (TRAP) as an effective method for molecular characterization of Pelargonium was demonstrated by Palumbo et al. (2007). They evaluated 46 accessions as one or two primer combinations generated enough bands and they were highly polymorphic. The two dendrograms generated from two different data sets were similar suggesting that only a few TRAP amplifications could be enough to generate sufficient number of markers to classify Pelargonium accessions.

Chimonanthers praecox, endemic to China, is being in cultivation for more than 1000 years as garden, potted and cut flower plant. Many cultivars have been developed during its long history of cultivation. The identification and genetic relationship of these resource were studied based mainly on morphological traits. In a study by Zhao et al. (2007), ISSR and RAPD markers were used to characterize 72
clones from two regions and reported that either ISSR or RAPD should be sufficient to distinguish all the clones surveyed.

Nine genotypes of marigold from two species were characterized through electrophoresis of protein and RAPD markers by Mor et al. (2008). The dendrogram based on protein electrophoresis grouped the nine genotypes in two clusters species wise whereas, RAPD analysis showed clear cut genotypes and species differences. These results confirm the reliability of RAPD markers over protein electrophoresis.

Lu et al. (2011) used Inter Simple Sequence Repeat (ISSR) markers to assess the genetic diversity and population structure of 151 Cymbidium sinense cultivars collected from China and Japan. One fifty one cultivars were clustered into seven main groups and approximated their geographical distribution. Population structure analysis revealed six subpopulations generally consistent with neighbor joining clustering suggesting that collection and in situ conservation are important for conservation and genetic improvement.

RAPD based genetic diversities have been correlated with three morphological traits (petal colour, flower diameter, blooming period) in 15 Camellia cultivars by Wang et al. (2011). They divided 15 cultivars into two groups using cluster analysis. A correlation was found between RAPD markers and petal colour in first group and not for flower diameter and blooming period. They opined that the RAPD information is useful in identification, classification, phylogenesis and breeding of Camellia cultivars.

The genetic diversity in six wild Passiflora species was determined by morphological and molecular characteristics by Viana et al. (2010). The cluster analysis using morphological data showed three groups where as the dendrogram constructed using RAPD showed six different groups. They reported that the species showed high morphological and molecular inter and intra specific variability.

Alejandro et al. (2007) performed both ISSR and AFLP analysis for molecular identification of new varieties of Nierembergia linariaefolia (a native Argentinean ornamental) belonging to Solanaceae family in six clones. They found both ISSR and
AFLP techniques suitable for monitoring genetic diversity. However ISSR was found a better choice than AFLP.

Genetic relationship of 51 ornamental peach was determined using AFLP markers by Hu *et al.* (2005). A total of 265 out of 275 useful markers ranging in size from 75 to 500 base pairs were generated using six Eco RI / Mse I AFLP primer pairs. They were polymorphic and 18 out of 20 upright ornamental peach formed a group indicating cultivars with upright growth habit had close genetic relationship.

RAPDs were used to assess the level of genetic variations among the cut flower *Anthurium* with eight decamer primers. The average genetic similarity of 91.34 percent indicated significant low genetic distance and thus, low genetic variations among the cultivars (Nowbuth *et al.*, 2005).

An AFLP strategy was developed by Zhang *et al.* (2008) in 13 modern rose for variety identification purpose with 12 pre screened primer combinations to differentiate close varieties or varieties showing no morphological difference.

AFLP analysis using 17 primer combinations was carried out by Loh *et al.* (1999) on two species of *Caladiums*. They distinguished these two species by their unique and different banding patterns. They suggested that they could be used to characterize and identify the species for registration and for future breeding programme and systematic.

Rajaseger *et al.* (1997) have optimised the genomic DNA extraction from fresh and dry samples of lamina and corolla lobes of *Ixora* sp. They precipitated DNA with phenol chloroform extraction to obtain high quality DNA suitable for consistent PCR amplification. Thus, RAPD profiles could be used for identification at taxa level. The cluster analysis from six primers grouped all 22 cultivars under two groups.

Horticulturists have shown increased interest in *Losepetalan chinense*, an attractive flowering plant, besides having desirable landscape characteristics *viz.* fast growing, tolerant to disease and pest and abundant flowering capacity. Due to rapid introduction and commercialization, there is confusion concerning the identity and distinctness of cultivars which could be solved by RAPD markers. This method
grouped the 14 introductions into four clusters and most named introductions were closely related to other unnamed introduction suggesting for possible exploration in breeding programmes (Gawel et al., 1996).

Studies were undertaken by Barika et al. (2006) for the identification and determination of genetic variation in two species and 16 varieties of Hibiscus through RAPD markers using ten primers ranging from 0.3 to 2.5 kb. Among the genotypes, 16 varieties and two species formed one cluster with two major subcluster and the genetic distance was very close within the varieties and among the species.

Kumar et al. (2006) detected the genetic variability among eleven radio mutants from two chrysanthemum cultivars. Twenty one primers out of 40 random primers reproduced (118 out of 156) polymorphic bands. The cluster analysis produced three major groups and opined RAPD markers can be a useful tool to supplement the distinctness, uniformity and stability analysis for plant variety protection.

Parab and Krishnan (2008) used ISSR and RAPD markers to assess the genetic variation in Rhynchostylis retusa, an epiphytic orchid from Goa. They recorded high level polymorphism in RAPD (76.13 %) than ISSR (62.6 %).

Fifteen commercial cultivars of rose were evaluated using 10 primers by Emadpour et al. (2009). Out of 126 bands produced, 73 were polymorphic bands. The dendrogram revealed two main clusters with subgroups which showed relatively considerable genetic diversity.

The genetic diversity and characteristics of commercial interest of Passiflora species make it useful to characterise wild germplasm because of their potential use for fruit, ornamental and medicinal purposes. In this regard, Silva et al. (2010) evaluated genetic diversity using RAPD markers in 322 genotypes of Passiflora cincinnata and observed wide variability after thirteen primers generated 95 polymorphic and one monomorphic band. In Clitoria ternatea, Swati et al. (2011) observed that the genomic sequences of 4 genotypes did not cluster together even though the origin was same for all the genotypes when they used RAPD markers.
The molecular analysis using RAPD markers revealed moderate variations within species in 4 species of *Senesio* belonging to asteraceae family. 191 polymorphic bands out of 228 bands using 12 primers resulted in two major groups based on geographical distribution (Sumangala *et al.*, 2011).

To investigate the genetic diversity of *Rosa* accessions Mirzaei and Rahmani (2011) used RAPD approach. Nine of ten primers amplified 138 bands with 111 polymorphic bands (80 %) and DNA fragments ranged from 250 to 6000 bp. Genetic similarities between *Rosa* cultivars ranged from 0.42 to 0.84 and the dendrogram revealed two main clusters, revealing considerable genetic diversity among these cultivars.

Singh and Malik (2012) assessed the genetic diversity in Golden Cron Beard (*Verbesina encelioides* of Asteracceae family) – an ornamental plant, grown widely in gardens. In the study, the RAPD analysis has generated 73 polymorphic bands out of total 97 clear bands. A dendrogram constructed based on UPGMA clustering method revealed 2 major groups. This study highlights that the high genetic diversity within a species which could be attributed to wide distribution and divergent ecological conditions.

A genetic analysis was performed on a population derived from crosses between ornamental *Viburnum lantana* and *Viburnum carlesii* (Al-Niemi *et al.*, 2011). Linkage maps were developed using AFLP, RAPD and STSM. These maps were used to determine the location of several major genes influencing bud colour, flower scent and resistance to leaf spots and *Verticillium*.

Genetic diversity was evaluated by RAPD markers on morpho-agronomic characters for 42 accessions of *Gerbera*. A total of 74 polymorphic bands were obtained employing a set of 12 primers. The genetic structure revealed six clusters. Jaccard and Shanon index indicated the presence of higher genetic variation among commercial accessions compared to non-commercial accessions (Mata *et al.*, 2009).

**2.8. Molecular markers in Olives- an important genera of Oleaceae**

RAPD analysis of olive (*Olea europaea* L.) cultivars was carried out by Fabbri *et al.* (1995). Seventeen olive cultivars, including oil and table olive cultivars
originating from throughout the Mediterranean area, were screened using RAPD markers. The results indicated a high degree of polymorphism in the germplasm examined. Forty random decamer primers were screened. Seventeen of these produced 47 reproducible amplification fragments useful as polymorphic markers. Each of the 17 cultivars was discriminated with a few primers. Results were analysed for similarity among the cultivars and a cluster analysis was performed. These analyses revealed two main groups, one comprising primarily small-fruited cultivars grown mainly for oil production and the other characterized by having large fruit. There was no apparent clustering of olive cultivars according to their geographic origins.

Another attempt on molecular characterization of olive varieties using RAPD markers was carried out by Wiesman et al. (1998). Primers were identified and used in combination to discriminate between different varieties. Significant biodiversity was demonstrated among ‘Nabali’ olive trees, suggesting that the grouping known as ‘Nabali’ is actually a mixture of genetically distinct variants. On the other hand, RAPD profiles of selected variants of ‘Souri’, revealed a high degree of similarity, indicating that these variants represent environmental phenotypes of the same genome. Molecular differences were demonstrated between the ‘Nabali’ group variants and ‘Souri’, while recently developed or introduced varieties showed individually distinct RAPD profiles.

Forty olive cultivars from Valencia, Spain were screened using RAPD markers. Unweighted pair group method cluster analysis of their similarity values revealed two main groups divided according to geographic origin within Valencia and a third group which included two Spanish cultivars from regions outside of Valencia was clustered separately as reported by Cortes et al. (2001).

A study was conducted by nuclear mitochondrial DNAs of cultivated and wild olives using RAPD and RFLP markers for differentiating two groups in the wild forms: the Western tru oleaster and the feral forms, which is not recognizable based on morphology by De Caraffa et al. (2002). They opined further that the study of local varieties is important to understand the extent of gene flow between wild and cultivated forms and to justify the preservation of the biodiversity.
Belaj et al. (2002) studied the genetic diversity and relationship using RAPD technique in 103 olive cultivars from World Germplasm Bank in Spain and detected high genetic differentiation among cultivars within each Mediterranean zone and a low variability between zones.

Kockar and Ihker (2003) modified the genomic DNA purification and optimized AP-PCR parameters for olives. They reported that this technique can be used reliably to generate useful DNA fingerprints of olive cultivars. It was possible for them to differentiate various olive cultivars using three different primers and analysis of the finger profiles.

In another study, the thirteen RAPD primers were assayed in 82 Spanish olive germplasm for characterization and identification of cultivars of economical interest (Belaj et al., 2004) for generating a molecular database to catalogue the cultivars. Their ANOVA analysis indicated that most of the genetic variability was attributed to differences of cultivations within each zone.

Sesli and Yegenogu (2009) studied the genetic similarities and distances among 16 wild olive by RAPD–PCR techniques and reported high polymorphism. Further, they opined that the results could be used to determine the origins of cultural sub varieties.

2.8.1. Use of AFLP

The first linkage map of the olive genome, using RAPD and AFLP as dominant markers and RFLP and SSR as co-dominant markers was constructed by Rosa et al. (2003) in 95 individuals of cross progeny derived from two highly heterozygous olive cultivars. They reported that both AFLP and RAPD markers were homogeneously distributed in all the linkage groups reported.

Hagidikitiou et al. (2005) developed a reliable reference data base to discriminate major Greek olive cultivars and to reveal their genetic relationship through AFLP and RAPD markers and morphological traits. Morphological characters showed a very low correlation with the molecular date. They observed that
olive cultivars got clustered according to fruit size but not according to geographical origin.

Rao et al. (2009) studied the molecular diversity and genetic relationships of southern Italian olive cultivars by AFLP and morphological traits in 70 genotypes. AFLP profiling provided clear genetic differentiation between cultivars. Morphological and AFLP based genetic distances yielded different hierarchical patterns. Although the two data sets were both useful for assessing the presence of genetic variation, there was no good correlation between genetic distances estimated using AFLPs and morphological markers.

2.8.2. Use of Microsatellites (SSR)

Rosa et al. (2004) used microsatellites for paternity testing of olive progenies to distinguish 23 olive cultivars. The results showed that the short DNA protocol and the highly polymorphic nature of the microsatellite analysis is a convenient technique to assess the routine crosses used in breeding programs and to check self incompatibility in olive. The best characteristic of primers used for paternity testing was highly polymorphic with respect to potential rogue parents though it did not show with parents.

Nine SSR primers were assayed by Belaj et al. (2004) in 35 Spanish an Italian olive cultivars for characterization an identification. Only 3 SSR primer pairs made possible the identification of all cultivars.

Genetic evidence of intra cultivar variability within Iberian olive cultivars was shown by Lopes et al. (2004). A collection of 130 samples originating from diverse areas in Europe and corresponding to 67 different cultivar denominations were genotyped at 14 microsatellite loci. In total, 135 alleles with a mean number of 9.6 alleles per locus were detected. The existence of homonyms or mislabeled samples in olive germplasm collection was evidenced by allele differences up to 60 % between samples of the same denomination.

Noormohammadi et al. (2007) screened 92 accessions belonging to 10 main olive cultivars by 13 microsatellite markers revealing high genetic variability both
within and between cultivars. Totally 72 alleles were detected with a mean number of 5.5 alleles per locus. Twenty four unique allelic patterns were observed and six genotypes showed 15 unique alleles.

Molecular characterization and identification of 20 Tunisian olive varieties using 10 SSR markers was done by Rekik et al. (2005). They found SSR markers extremely useful for addressing the issue of homonymy and synonymy in cultivars. They were able to establish that the cultivar “Chetoui” is very likely to be derived from a single clone and others showed some degree of heterozygosity.

2.8.3. Chloroplast DNA variations:

Intieri et al. (2007) analysed 13 cultivars and one feral accession of olive for polymorphism to identify simple and reliable PCR markers useful for cultivar identification and oil traceability. They used RFLPs of the PCR products and sequencing of the intergenic spacer of chloroplast DNA. The chloroplastic markers analysed showed a pattern of variation sufficient to discriminate four out of 13 cultivars analysed.

Besnard (2008) analysed 18 olive trees displaying various cp DNA haplotypes belonging to six olive subspecies. Amplification of the intergenic trn D-trn T spacer was performed using the primers trn D-F and trn T-R and PCR was conducted in 50 µl reaction and detected relatively low variation between distant populations.

2.9. Errors encountered in RAPDs

A study was conducted to know the factors influencing RAPD band patterns in Malus domestica (Yae et al., 1995). This study was carried out to investigate several factors affecting RAPD band patterns in apple and to establish the optimum RAPD conditions. The optimum composition of PCR reaction solution for RAPD assay of Malus domestica [M.pumila] was 10 ng template DNA, two units Taq polymerase, 10 ng primer, 50-100 µM dNTPs, 2.5µl 10X reaction buffer. Fifty cycles of amplification produced best RAPD band patterns.

Staub et al. (1996) reported the sources of potential errors in the applications of RAPD’s in cucumber. The influence of tissue age, pathogen infestation, intra
population contamination and PCR conditions were assessed as sources of error in RAPD analysis. DNA from young, uninfected tissue from cucumber (lines G421 and H-19) leaves provided the most consistent results. Cucumber plants infected with *Sphaerotheca fuliginea* showed variation in RAPD banding patterns compared with those of uninfected plants. Differences in banding patterns were detectable when DNA from the lines was mixed at dilution ratios of 20:1 but not 50:1. Differing lots of commercially available 10X reaction buffer, MgCl$_2$ stock solutions and *Taq* DNA polymerase affected RAPD banding patterns and overall yield. For reproducibility of RAPD assays, it may be necessary to optimize reactions for specific lots of PCR reagents from either commercial or in-house sources.