1. INTRODUCTION

Plants which are used in medicine are usually referred to as herbals. In India about 2500 plant species belonging to more than 1000 genera are used by traditional healers and about 500 plant species are utilized by 159 different pharmaceutical companies (Chandel et al., 1996). In the recent days, Researches in the development and application of in vitro techniques for the conservation of the plant germplasm have drastically increased. In future, conservation and biotechnology will be able to contribute significantly to the sustainable management of the endemic and/or economically important plant species.

Interestingly enough, nearly 95% of the medicinal plants are harvested from the wild. The populations, urbanization, habitat degradation, introduction of exotic Spss, shrinking forests, over harvesting and related factors have brought several medicinal plants to the very brink of extinction. Henceforth, Conservation of the threatened medicinal plants has become the most important factor of all the nations, especially the biodiversity-rich nations.

Plant is normally considered as a source of medicine. It is of paramount importance in the countries like India and Sri Lanka which have well developed traditional systems of medicine called Ayurveda, Siddha and Unani, all of which derive more than 90% of medicament from the higher plants. Several of these also constitute important ingredients of allopathic medicines. Most of the requirements of the medicinal plants are obtained from wild sources. But, owing to changing ecological factors in the forest and unscrupulous collection of medicinal plants, the wealth of medicinal plant is getting depleted and many of them have already become extinct. The compendium of these plants from the forest cannot cope up with the ever increasing and changing demand from the pharmaceutical, perfumery and cosmetic industries. Biotechnology, with its apparently limitless potential, offers new and exciting opportunities to address the myriad problems in the field of medicinal plant cultivation. Some of the important applications of biotechnology in medicinal plants are rapid clonal propagation to generate good quality planting material, exploiting somoclonal variation and genetic engineering technique for the crop improvement, in vitro selection for resistance to biotic and abiotic stress, in vitro conservation and safe exchange of germ plasm and secondary metabolite production (Pushpangadhan, 1992).
Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First and foremost, it is likely that these phytochemicals would find their way into the arsenal of antimicrobial drugs prescribed by physicians several are already being tested in humans (Clark, 1996). After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is very much limited. Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993 (Alper, 1998). New sources, especially plant sources are also being investigated. Second, the public is aware of the fact that the problems with the over prescription and misuse of traditional antibiotics. Besides, many people are primarily interested in having more autonomy over their medical care. A multitude of plant compounds is readily available over the counter from the herbal suppliers and natural food stores, for self-medication with these substances in common place. The uses of plant extracts, as well as other alternative forms of medical treatments, have attained great popularity in the late 1990s. Earlier in this decade, approximately one-third of the people surveyed in the United States had at least one "Unconventional" therapy during the previous year (Eisenberg et al., 1993). It was reported that in 1996, sale of herbal medicines increased 37% over that in 1995 (Klink, 1997). It is speculated that the American public may be reacting to over prescription of sometimes-toxic drugs, just as their predecessors of the 19th century who reacted to the overuse of medicines for bleeding (Yankauer, 1997).

Tissue culture technique can be used to induce shoot formation either directly or indirectly by inducing callus formation and regeneration of shoots or roots from the callus. Apparently, Tissue culture is a modern biotechnological innovation for rapid propagation of a large number of uniform plants. Application of this method is the novel option to get rid of insurmountable problems. In a nutshell it has become a linch-pin to find out many solutions for the stringent biological constraints and environmental uncertainties. Considerable effort might lead to the production of high yielding improved plant types, production of secondary metabolites under in vitro condition and to conservation of threatened and endangered species. It is vitally important for the in vitro conservation of plant germplasm and also vital supportive technology for long-term cryopreservation based storage. Conservation of plants can itself be a goal of in vitro plant cell and tissue culture programmes (Feijoo and Iglesia, 1998).

Micropropagation is a general term which acutely describes a variety of routes, for the propagation of selected germplasm using in vitro techniques. Micropropagation of medicinal plants under controlled germ free conditions enable enormous and fast
multiplication and ensures the availability throughout the year irrespective of the external environment. This is especially used in the case of the rare and endangered plants. Clonal propagation of elite plants is a viable technology in plant tissue culture. Several industries have standardized protocols for the multiplication of ornamental plants such as Gerbera, carnation and roses and plantation crops such as banana and cardamom (Pierik, 1991). Medicinal plants are the focus of attention to the researchers in the field of biotechnology as many of the drug industries depend solely on plants for the production of valuable compounds (Kareem, 1997; Shanker et al., 1997).

1.1 Legume Family (Fabaceae)

The legume family (Fabaceae) is the third largest family of flowering plants, with approximately 650 genera and nearly 20,000 species (Doyle, 1994). Its species range from large tropical canopy trees to small herbs found in temperate zones, humid tropics, arid zones, highlands, savannas, and lowlands. The Fabaceae contains many taxa of industrial or pharmaceutical importance. Legume seeds constitute the second most important plant source of human and animal food (Vietmeyer, 1986). Other new products would include new food sources, but the majority would provide industrial products such as dyes from indigo, fiber pulps, vegetables and pharmaceutical products. Many legumes contain organic chemicals in sufficient quantity to be economically useful as feed stocks or raw materials for many scientific, technological and commercial applications. Legumes can biologically fix nitrogen, adding annually up to 500 kg N/ha/year to the soil. Not only do other legume species provide hope for combating food shortages in developing countries, but also they do provide many speciality products such as rotenoids (Balandrin et al., 1985) for use as pesticides in developed countries.

1.2 Clitoria ternatea

*Clitoria ternatea* L. belongs to the family Fabaceae and is distributed in tropical Asia, Philippines Islands and Madagascar. It is an ornamental perennial climber with conspicuous blue or white flowers and in India, it is commonly called butterfly pea (Anonymous, 1988). It is a highly palatable forage legume, generally preferred by live stock over other legumes. It is also used as a cover crop or green manure. The root has been used in the treatment of various ailments like indigestion, constipation, arthritis and eye ailments. It is also employed in cases of ascetics, enlargement of the abdominal viscera, sore throat, skin diseases, etc (Anonymous, 1988; Morris, 1999). The root, stem and flower are highly recommended for the treatment of snake-bite and scorpion-sting. The extract of *C. ternatea* was found to have anxiolytic, antidepressant, anticonvulsant and antistress properties (Jain and Basal, 2003). The United State Department of Agriculture is dedicated to conserving *C. ternatea* along with other leguminous species with potentially
useful phytochemicals (Morris, 1999). However, pharmaceuticals companies largely depend upon material harvested from naturally occurring stands. Due to unrestricted large-scale exploitation of these natural resources, coupled with limited cultivation and insufficient attempts for its replenishment, the wild stock of this species has been markedly depleted, so now it is listed as a rare species by the International Union for Conservation of Nature and Natural Resources (IUCNNR) (Panday et al., 1993).

Plate 1: *Clitoria ternatea* Linn.

1.3 Scientific Classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Sub Family</td>
<td>Faboideae</td>
</tr>
<tr>
<td>Tribe</td>
<td>Cicereae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Clitoria</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>ternatea</em></td>
</tr>
<tr>
<td>Binominal Name</td>
<td><em>Clitoria ternatea</em> L.</td>
</tr>
</tbody>
</table>

1.4 Origin and Distribution

Butterfly pea is supposed to have originated in tropical Asia. The true origin is obscured by extensive cultivation and naturalization around the globe. Butterfly pea has been widely distributed in many tropical and subtropical countries namely South and Central America, East and West Indies, China and India. It has been grown as a persistent perennial. The flowers are used to give a blue tinge to rice cakes and boiled rice. The
young pods may be consumed like string beans. Leaves are also used to dye food or are eaten as a pot herb.

1.5 Plant Descriptions

Scientifically, butterfly pea (C. ternatea L.) belongs to the family Fabaceae and subfamily papilionoideae and it has a variety of recognized names. There are at least 12 other species recognized in this genus. They are C. albiflora, C. bracteata, C. coelestris, C. parviflora, C. pilosula, C. purpurea, C. ternatensium, Lathyrus spectabilis, Ternatea ternatea, and Ternatea vulgaris. C. ternatea and C. purpurea are partially domesticated and may have potential for forage use.

The butterfly pea is a 90 to 162 cm tall, long-lived perennial herb with a climbing habit (Kalaimani and Michael Gomez, 2001). Its flowers are blue scabbards linear and flat, 6-12 cm long (Kalaimani and Michael Gomez, 2003) similar to those of beans. The thick horizontal root, which may grow to more than 2 m long, bears one to several purplish, glaucous, wiry stems. The leathery leaves consist of three-five leaflets. Clitoria has chasmogamous (insect pollinating) and cleistogamous (self-pollinating) flowers. Flower colour, position and structure vary from species to species (Plate 1).
Table 1: Agronomic and Biochemical characteristics of *Clitoria ternatea* Linn.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>7 to 8</td>
</tr>
<tr>
<td>Soil fertility</td>
<td>Average to low</td>
</tr>
<tr>
<td>Drainage</td>
<td>Does not tolerate saturated soils</td>
</tr>
<tr>
<td>Altitude above sea level</td>
<td>0-1600 m (0-5,249 ft)</td>
</tr>
<tr>
<td>Precipitation</td>
<td>800 mm (31.5 in)</td>
</tr>
<tr>
<td>Planting rate</td>
<td>Monoculture : 20-25 kg/ha (18-22.5 lb/ac) mixed with grasses: 10-15 kg/ha (9-13.5 lb/ac)</td>
</tr>
<tr>
<td>Planting depth</td>
<td>&lt;2 cm (&lt;0.8 in)</td>
</tr>
<tr>
<td>Fertilization at planting</td>
<td>40 kg N/ha (36 lb/ac)</td>
</tr>
<tr>
<td>Crude protein content</td>
<td>18%-24%</td>
</tr>
<tr>
<td>Digestibility</td>
<td>60% - 75%</td>
</tr>
<tr>
<td>Management</td>
<td>Cutting and rotational grazing when associated with grasses</td>
</tr>
<tr>
<td>Stocking rate</td>
<td>2500 kg LW/ha</td>
</tr>
<tr>
<td>Seed rate</td>
<td>2-4 kg/ha for permanent pastures 6 kg/ha for short-term phase pastures</td>
</tr>
<tr>
<td>Spacing</td>
<td>15-30 cm (Narrow row spacing preferred)</td>
</tr>
<tr>
<td>Sowing depth (moist soils)</td>
<td>2.5 – 6.5 cm</td>
</tr>
</tbody>
</table>

Table 2: Amino acid composition as percent of crude protein (Barro and Ribeiro, 1983)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Arg</th>
<th>Cys</th>
<th>Gly</th>
<th>Hys</th>
<th>Lis</th>
<th>Leu</th>
<th>Lys</th>
<th>Met</th>
<th>Phe</th>
<th>Thr</th>
<th>Try</th>
<th>Tyr</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>7.4</td>
<td>2.5</td>
<td>4.1</td>
<td>2.4</td>
<td>4.2</td>
<td>7.4</td>
<td>6.1</td>
<td>1.0</td>
<td>3.6</td>
<td>2.2</td>
<td>1.2</td>
<td>3.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>
1.6 Chemical Constituents

Major flavonol glycosides, 3-O-(2''-O-alpha-rhamnosyl-6''-O-malonyl)-beta-glucoside, 3-O-(6''-O-alpha-rhamnosyl-6''-O-malonyl)-beta-glucoside and 3-O-(2'',6''-di-O-alpha-rhamnosyl)-beta-glucoside of kaemferol, quercetin and myricetin were isolated from the petals (Kogawa et al., 2006; Terahara et al., 1996). The flowers also contain minor delphinidin glycosides, 3-O-b-glucoside, 3-O-(2''-O-a-rahmnosyl)-b-glucoside, 3-O-(2''-O-a-rahmnosyl-6''-O-malonyl)-b-glucoside of delphinidin (Kogawa et al., 2006). Eight anthocyanins (ternatins C1, C2, C3, C4, C5 and D3, and preternatins A3 and C4) were also isolated from the flowers (Terahara et al., 1996; Terahara et al., 1998). Six ternatins from the flowers were partly characterized as highly acylated dephinidin derivatives. Deacylternatin was determined as delphinidin 3, 3’, 5’-tri-O-b-D-glucopyranoside (Terahara et al., 1990). White petals do not contain anthocyanins. There are low levels of condensed tannins (0-2.48 mg catechin/g) and protein precipitable polyphenols (0.16-0.77 mg tannic acid/g) in the raw mature seeds (Laurena et al., 1994). The seeds contain a highly basic small protein named finotin (Kelemu et al., 2003). Clitoria ternatea flowers contain little calcium (1.9 mg/100 g) compared to common vegetables as determined via inductively coupled plasma atomic emission spectroscopy (Chin et al., 1992).

1.7 Traditional Uses

C. ternatea is used as a brain tonic to promote memory and intelligence (Gomez and kalamani, 2003). The plant extract is used in a rejuvenating recipe to treat neurological disorders and is considered to be whole some for the intellect (Rai et al., 2002). Tribes use the root not only to induce abortion and but also to reduce abdominal swellings, sore throats and mucous disorders (Parimaladevi et al., 2003). The juice of the root is mixed with cold milk and is drunk to remove phlegm and for chronic bronchitis (Parimaladevi et al., 2004).

The roots are bitter, refrigerant, laxative, diuretic, anthelmintic and tonic and are useful in dementia, hemicrania, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever while the leaves are useful in otalgia and hepatopathy (Jain and Basal, 2003). The plant is considered to be very useful for eye infections, skin diseases, urinary troubles, ulcers and has antidotal properties (Gomez and kalamani, 2003).
1.8 Effects on Central Nervous System

*C. ternatea* has a wide spectrum of central nervous system activities i.e. nootropic, anxiolytic, anti-stress, antidepressant and anti-convulsant. *Clitoria ternatea* methanolic extract showed nootropic effects (facilitation of intellectual performance, learning and memory) as it decreased the time required for rats to occupy the central platform in the elevated plus maze and increased the discrimination index in object recognition tests. The plant exhibited weak anxiolytic activity by increasing the occupancy of rats in the open arm of the exploratory maze and the lit box of the light/dark exploratory test, and antidepressant activity as it decreased the immobility time in the tail suspension test. The methanolic extract reduced stress-induced ulcers and decreased the convulsing actions of pentylenetetrazol and maximum electroshock. Cognitive abilities were improved without the production of sedation and behavioral toxicity (Jain and Basal, 2003). Oral intubation of rats for 30 days with the aqueous root extract (100 mg/kg) paved the way to improve learning and memory. In neonatal and young adult rats, this led to significant increases in acetylcholine content in the hippocampus, pointing to a neurochemical basis for the improvement in learning and memory. The memory enhancing property of the root extract was also shown by its ability to improve retention and spatial learning performance in behavioral tests (Rai *et al.*, 2002). Alcoholic root extracts (300 & 500 mg/kg doses orally) were more effective than the aerial parts in attenuating memory deficits in rats and this was associated with increased levels of rat brain acetylcholine and acetyl cholinesterase (Taranalli *et al.*, 2000; Howes *et al.*, 2003). Relationships of these effects with inhibition of acetyl cholinesterase activity were not established, cortical acetyl cholinesterase activity was actually found to be increased cited in (Howes *et al.*, 2003). There was also an increase in the functional growth of the neurons of the amygdala (Rai *et al.*, 2005).

1.9 Anti-inflammatory, Analgesic and Antipyretic Activities

The methanol root extract (200-400 mg/kg) was given orally with the intention of reducing normal body temperature and yeast-induced pyrexia in rats in a dose-dependent manner. The antipyretic effect of the extract was comparable to that of an oral dose of paracetamol (150 mg/kg). Rat paw edema induced by carrageenin and vascular permeability induced by acetic acid were inhibited by both the doses of the methanol extract. The extract also markedly reduced the number of writhing responses in the acetic acid-induced writhing response test. Anti-inflammatory, analgesic and antipyretic activities of the plant were attributed to its flavonoid content (Parimaladevi *et al.*, 2004; Parimaladevi *et al.*, 2003).
1.10 Other Activities

The seed extract contains antifungal proteins which were similar to plant defensins previously characterized from radish seeds and gamma thionins from Poaceae seeds. A highly basic small protein, finotin, was also isolated from the seeds. This protein has broad and potent inhibitory effects on the growth of important plant fungal pathogens and the common bean bacterial blight pathogen, *Xanthomonas axonopodis* PV. Phaseoli (Kelemu *et al.*, 2004). Finotin also has insecticidal properties as it is a powerful inhibitor of two bean bruchids. The flatulence potential of this plant is the highest in comparison with hyacinth bean (*Dolichos lablab*), sabawel (*Mucuna pruriens*), lima bean (*Phaseolus lunatus*), swordbean (*Canavalia gladiata*), rice bean (*Vigna umbellate*) and jack bean (*Canavalia ensiformis*).

1.11 Toxicities

*C. ternatea* was shown to be non-toxic and non-bloating to livestock (Gomez and kalamani, 2003). *C. ternatea* did not produce sedation or behavioral toxicity (Jain and basal, 2003). In the acute toxicity test for determination of LD$_{50}$, the extract was found to be safe in animals even at a dose of 3.2 g/kg (Parimaladevi *et al.*, 2004).

1.12 *In vitro* Propagation

In the midst of different techniques of biotechnology, plant tissue culture is the one being applied in crop improvement programme. There is an increasing awareness of the potential application and limitation of plant cell and tissue culture technology for the production of novel genotypes with valuable attributes. In *C. ternatea* leaf explants showed shoot regeneration accompanied by callus formation. MS medium fortified with auxins (NAA or IAA) and BAP (0.5 mg/L) included a large number of multiple shoot buds directly from the young shoot tip explants of *C. ternatea* (Gomez and kalamani, 2003). The MS medium developed for micro propagation of *Clitoria* spp appears to be more advantageous for achieving increased number of multiple shoots. Formation of multiple plantlets from shoot tip culture could be of practical application for raising hybrid seedling of difficult crosses and mutagenesis *in vitro*. Regeneration of multiple plantlets from shoot tip explants on simple medium may be used for the mass production of plants, storage and maintenance of germplasm.
1.13 Medicinal Value

*C. ternatea* Linn, (Fabaceae) is also known as Aparajitha in India a flowering plant that insects pollinated. It is a vigorous, strongly persistent, herbaceous perennial legume. Its origin lies in south-east Asia and widely distributed in South Africa, Tanzania, Mauritius, and Cameroon. It has been used in the indigenous system of medicine for cooling, acrid, purgative, diuretics, laxative, anthelmintic, anti-ulcer properties. In animal tests, the methanolic extract of *C. ternatea* roots demonstrated anxiolytic, antidepressant, antistress and learning enhancing activity (Rai* et al.*, 2002).

1.14 Ornamental value

Among the several species of *Clitoria, C. ternatea and C. purpurea* have attractive flowers. *C. ternatea* has creamy blue & white coloured flowers which are solitary and very attractive. Because of their attractive nature of the flowers, they are valued as an important ornamental crop for the garden lovers. The hybrids between *C.purpurea* and *C. ternatea* produced intermediate coloured flowers and flower size was bigger when compared with parents. In the segregating progenies (F2) of above said crosses variation in flower colour was noticed viz., light pink colour, cream colour with blue borders, medium blue, dark blue with velvety appearance, violet, dark violet,besides the parental colours (Gomez and kalamani, 2003). For ornamental purpose, medium height segregants with attractive flower such as deep violet, light pink and velvety blue with less numbers of leaves can be exploited.

Health is wealth, according to an old adage. The WHO has defined health as not only the state of complete physical, mental and social well-being but also freedom from diseases or any infirmities. The overall health status of any population may serve as an index of its prosperity and welfare. The earth, as it is today, has turned out to be a hostile environment to live and it is heartening to note that 16% of the world population is challenged with serious health care problems. Protection against various diseases is therefore imperative. India has the distinction of hosting about 16% (>1 billion) of the world population and records 20% of world's mortality and 22% morbidity. These staggering figures call for measures towards health care.

Infectious diseases are still persisting as major health problem and nearly about 11 million children die each year. The worst affected are the developing countries, which accounts for nearly 99% of the mortality due to infectious diseases (Dipika Sur* et al.*, 2004; Nongkynrih* et al.*, 2004). In India, 7 million infectious disease deaths (of which, two million deaths are observed mostly in children and young adults
every year) account for 70% of infectious disease deaths and 13% of all deaths worldwide. The most frequently reported diseases are Leptospirosis, acute dysentery, typhoid fever and acute hepatitis (Jacob John et al., 2004). Such negative health trend calls for a renewed interest in prevention and treatment of infectious diseases. Though plenty of antibiotics are readily available, increasing incidence of resistance to antibiotics particularly in gram-negative bacteria (Alonso et al., 2000; Sader et al., 2002) has urged the researchers to look for viable and sustainable alternatives. It is evident that the Indian people have an unquenchable thirst for medicinal plants (over 3000 plants have been used indigenously) and use them for a wide range of health related applications from a common cold to memory improvement and treatment of poisonous snake bites to a cure for muscular dystrophy and the enhancement of body's general immunity (Pearce and Purushothaman, 1992). But, on the other hand, it is observed that the plant biodiversity has not been conserved and protected and also less explored against new diseases.

The alternate approach to drug discovery is through the medicinal plants. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. It is documented that 80% of the world population has faith in traditional medicine, particularly plant drugs for their primary health care. India is a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the ‘botanical garden’ of the world. India formally recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are used as traditional, folk and herbal medicine representing about 75% of the medicinal needs of the third world countries. In 1991, 42 new agents were introduced to medical practice of which 16 were natural products or were derived from natural products. Similarly in 1992, 4 new chemical entities were introduced and among them were natural products or their derivatives.

Medicinal plants also have the following benefits and aid in drug discovery.

- Plants are perennial sources of wide variety of novel drug molecules (chemical discovery). Example: Alkaloids, terpenoids, lignans, glycosides etc.
- Natural products serve as lead molecules for the development of many popular drugs.
- Herbal drugs have lesser side compared to other classes of synthetic drugs.
- Possibility of multidrug / target therapy.
Isoenzymes (isozymes) are the most valuable tools for the study of genetic variability within the population of plants and animals. Isozymes resolve all queries related to population biology, conservation biology and ecology as well.

The term isozyme is used for the reference to multiple forms of an enzyme with similar or identical catalytic activities occurring within the same organism. Isozymes may differ in their primary structure because they are encoded in different genes. The genetics of isozymes is well understood and thus isozymes can be used as effective markers in developmental genetics and in studies on differentiation.

Analysis of plant and animal genome, using molecular markers is proving valuable in breeding programme to rapidly develop the improved crops and livestock strains with enhanced productivity. These genomic studies are enabling scientists not only to gain valuable insights into how the crop and livestock genomes are organized but also to provide multitude of practical applications such as variety identification through DNA fingerprinting development of genetic maps which facilitate individual selection of economic trait such as disease resistance without cumbersome screening, cloning of important genes and evolutionary and phylogenetic studies (Strauss et al., 1988).

In recent years, isozymes have been used very successfully as biochemical marker in certain aspects of plant breeding and genetics as nearly neutral genetic markers (Chawla, 2002). Scandalios, (1975) distinguished the molecular markers in two classes: biochemical molecular marker derived from the studies of chemical products of gene expression and molecular genetic marker derived from direct analyses of polymorphism in DNA sequences. The use of isozymes as genetic markers has increased dramatically over the last decade as it has a number of important advantages over more conventional morphological markers.

Isozymes from soft plant tissues can be obtained from the macerates prepared in phosphate or Tris buffer. After gel electrophoresis, starch or acrylamide gels may be stained for several classes of enzymes. For esterases, the most commonly used substrate is 1% alpha-naphthyl acetate solution in 70% acetone, which is acted upon by many non-specific ester hydrolases. Peroxidases produce a distinctive colour reaction in the presence of hydrogen peroxide and certain phenolic compounds like benzidine and catechol (Brewbaker et al., 1968).

The basic of electrophoresis analysis of isoenzymes was laid down in 1957 (Stabbins, 1989). In 1959, Markert and Muller introduced the concept of isoenzyme, which they defined as the different molecular forms in which proteins might exist with the
same enzymatic specificity (Buth, 1984). This indicates that different variants of the same enzyme have identical or similar functions and are present in the same individual. As such, their importance for understanding gene action in development and differentiation was exploited during the 1960s in both the animals and plants.

In general, Isoenzymes, having common catalytic activity, may be widely synthesized under the control of different genes, active in different tissues and differing in molecular properties (Nash and Davis, 1975). Isozymic variations may also rise from allelic segregation at a single locus representing more subtle changes in the enzyme molecules. Isozyme analysis has been carried out in many plant species. These are peroxidase, catalase, amylase, leucine aminopeptidase, esterase, acid phosphatase, dehydrogenase, phosphorylase, transaminase and polyphenol oxidase, cytochrome oxidase etc (Rocha and Ting, 1971).

Molecular forms of an enzyme or isozyme can be separated by several biochemical methods including sedimentation, electrophoresis, chromatography and gel filtration and even serological methods. Of these, gel electrophoresis is basically a process of forced diffusion with in an electric field. In this, the proteins of the given sample are moved through a gel or paper or cellulose by using electrical gradient. Different proteins assume different charges, at different pH. Based on the molecular weight, they move on to the gel. This results in separation of different bands that can be stained and differentiated. Acrylamide gels are more useful and can withstand wide range of pH. They are also well known for optical clarity (Sako and Stahmann, 1972).

Nevertheless, isoenzyme played a minor role in research on plant biochemistry until 1966 when genetic polymorphism for isoenzyme within the same population was discovered (Stabbins, 1989; Wendel and weeden, 1989). That revealed the possibility for population genetics to make precise quantitative estimates of genetic variability based upon one parameter of the molecular structure of the primary product of the genes themselves.

For centuries, mankind has been solely dependent on plants as a source of carbohydrates, proteins and fats for food and shelter. In addition, plants are valuable sources of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Over 80% of the approximately 30,000 known natural products are of plant origin. The number of known chemical structures is estimated to be nearly fourfold greater than that in the microbial kingdom. In 1985, of the 3500 new chemical structures identified, 2600 came from the higher plants. Worldwide, 121 clinically useful prescription drugs are derived
from plants (Payne et al., 1987). Plants will continue to provide novel products as well as chemical models for new drugs in the coming centuries mainly because the chemistry of the majority of plant species is yet to be characterized. The advent of chemical analyses and the characterization of molecular structures have helped in precisely identifying these plants and correlating them with their activity under controlled experimentation. Despite advancements in synthetic chemistry, we still depend upon biological sources for a number of secondary metabolites including pharmaceuticals. Their complex structural features are difficult to synthesize (Ramachandra Rao and Ravishankar, 2002).

Elaborative pathways from basic primary metabolites, which are synthesized immediately as a result of photosynthetic activity, produce secondary metabolites. Many of them are unique to the plant kingdom and are not produced by microbes or animals. However, with the advancement of transgenic research, it is possible to produce compounds and molecules, which were also not originally synthesized in plants (Ramachandra Rao and Ravishankar, 2002).

About 20% (approximately 16000) of all known natural products are classified as alkaloids (Verpoorte et al., 1993). Many alkaloids are very important as plant-derived pharmaceuticals. Other natural products used medicinally are isoprenoids (mono, sesqui, di, triterpenoids, steroids, and cardenolides), quinones, lignans or other plant phenols such as flavonoids.

Phenolic phytochemicals are large group of substances that have been regarded as possible antioxidants. These may inhibit oxidative damage, mutagenesis, carcinogenesis, and thereby reduce the risk of chronic degenerative diseases associated with free radicals (Decker, 1995; Kelly et al., 2001). Flavonoids from a large family of secondary plant metabolites (poly phenolics), comprising anthocyanins, flavonols, flavones, catechins and flavonones, and many are present in plant tissues in relatively high concentrations as sugar conjugates (Harborne, 1986). Over 5000 different flavonoids have been described. The diversity in their chemical structure confers on them a wide range of biological activities. In plants, their function seems to be linked with protection against ultraviolet radiation, microbial invasion and both insect and mammalian herbivores (Sebestian et al., 2006). Flavonoids have a range of biological effects in ever so many mammalian cell systems, in vitro as well as in vivo. They have been suggested as antiinflammatory, antiviral and antiallergic agents, and may modulate a wide range of mammalian enzyme activities such as cytochrome P-450, epoxide hydrase, and glutathione transferase (Middleton, 1998; Ono and Nakane, 1990). They may also contribute to the maintenance of normal blood vessel conditions by decreasing capillary permeability and fragility (Brown, 1980; Mitscher
et al., 1996). Flavonoids are best known for their antioxidant properties, and may act in vitro as reducing agents, hydrogen donors, free radical quenchers and metal ion chelators (Shahidi and Wanasundara, 1992; Duthie et al., 1997). They have been shown to exert antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-inflammatory, antioxidant, anti-hepatotoxic, anti-hypertensive, hypolipidemic and antiplatelet activities (Formica and Regelson, 1995). It clearly exhibits beneficial effects on cardiovascular diseases (Middleton et al., 2000).

Genetic polymorphism is defined as the simultaneous occurrence of a trait in the population of two or more discontinuous variants or genotypes. The first DNA utilized markers were fragment produced by restriction digestion. The use of molecular marker is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purpose. A marker must be polymorphic in nature.

The prime principle is that by virtue of the short primer sequence, there is a high probability that two priming sites will occur in the genome in inverted orientation and in close proximity. The intervening region is amplified, resulting in different sizes of DNA fragments, which can be resolved by gel electrophoresis. The amplified DNA fragments behave as simple Mandolin markers. The main advantage of RAPD analysis is that no prior knowledge of DNA sequence is necessary to design the primer. For most plant species, a single primer (random sequence with at least 60% G+C% lacking inverted repeats) is predicted to generate an average of 2-10 amplification products. Each amplified product represents one allele per locus. RAPDs have been extensively used for a number of horticultural crops. They have been extensively used in variety identification, assessment of genetic diversity, genetic purity, sex determination and in tissue cultural studies.

RAPD analysis is a PCR based molecular marker technique. In this technique, single short oligonucleotide primers are arbitrarily selected to amplify a set of DNA segment distributed randomly throughout the genome. Two groups viz., reproducible and heritable DNA fragment (Welsh and McClelland, 1990) are independently discovered and amplified from the genomic DNA using only single sequence arbitrary primers. This method is termed random amplified polymorphic DNA (RAPD) (Williams et al., 1990). According to that short primers of arbitrary nucleotide sequences may be used to reproducibly amplify segments of genomic DNA from a wide variety of species. Polymorphism among the amplification products are detected frequently and are useful as genetic markers and can be detected through examination of an ethidium bromide stained agarose gel. This process described here, uses primers of arbitrary nucleotide sequence to
access random segments of genomic DNA to reveal polymorphism. These polymorphisms, simply detected as DNA segments, which amplify from one parent but not the other, are inherited in a Mendelian fashion and can be used to construct genetic maps in a variety of species. These RAPD markers are pronounced “rapid markers”.

RAPD are the result of amplification using, short sequence arbitrary design oligonucleotide (tenmer). Amplification is allowed to proceed by incorporating a low annealing temperature in the thermal cycling profile. The resulting amplification products are size separated on agarose gels. A DNA fragment pattern of low complexity is observed following ethidium bromide staining. When the same primer is used with DNA from different species, unique fragment patterns are observed. More importantly, when DNA from different strains from the same species is used, fragment patterns are nearly similar with the exception that an occasional amplified fragment is present in one strain and absent in another. It is determined that these fragment polymorphisms are heritable and constitute a new class of genetic markers. The frequency of polymorphism is observed is dependent upon the species studies, ranging from 0.2 to 1 polymorphism per primer in a selected angiosperm.

RAPD has proven to be extremely popular with plant scientists. A recent Bibliographic search identified over 2000 publications in which RAPD markers have been used. This popularity has been fueled by the simplicity of the method and the low capital investment required. No more than a thermal cycler and inexpensive horizontal gel electrophoresis equipment is needed. Also, low cost sets of random tenmer oligonucleotides may be purchased.

Most RAPD are amplification polymorphisms which are the result of either a nucleotide base change at one of the priming sites or an insertion/deletion event within the amplified region (William et al., 1990; Parks et al., 1991). DNA amplification from individuals heterozygous at RAPD marker loci generally is indistinguishable from the positive parent. Thus, RAPD markers are typically dominant markers although occasional co-dominant RAPD marker loci are observed as fragment length polymorphisms. RAPD markers have been used to construct linkage maps for several plants and also used to investigate genetic variations of natural populations and showed greater genetic variation between and within the populations.