CHAPTER II

REVIEW OF LITERATURE
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2.1. Zingiberaceae

Zingiberaceae, the largest family in the monocotyledon order Scitaminales, consists of perennial, mostly terrestrial, rarely epiphytic herbs with aromatic, fleshy, tuberous or non-tuberous rhizomes. Stem is a pseudostem with sheathing leaf bases. Leaves distichous, simple, those towards base of the plant usually bladeless and reduced to sheaths; leaf sheath open, ligule usually present, petioles present or not and located between leaf blade and sheath, leaf blade sub-orbicular or lanceolate or strap shaped with prominent mid vein and numerous lateral veins. Inflorescence terminally produced on pseudostem or on separate shoots; it is sometimes with bracteolate cincinni in bracts axils and then thyrse or some cases raceme or spike with bisexual, epigynous and zygomorphic flowers. Bracts and bracteoles present, coloured and conspicuous. Calyx is usually tubular, thin, split on one side, sometimes spathe like, apex 3 toothed or lobed. Corolla is tubular, distally 3 lobed with varying shape and size. Androecium contains two whorls of six stamens or staminodes, lateral 2 staminodes of outer whorl are petaloid or forming small teeth at the base of labellum or adnate to the labellum or absent, median staminode of outer whorl is always reduced. Labellum formed from lateral 2 staminodes of inner whorl. Fertile stamen is at the middle of inner whorl, filament long or short, anther locules 2 and introse, dehiscing by slits or by pores. Ovary inferior, 3 loculed at initial stages and becomes 1 or 3 loculed at maturity. Placentation is parietal, basal or axile, style 1 and stylodes 2, reduced to nectaries at the apex of ovary. Fruit is a
capsule, fleshy or dry, dehiscent or indehiscent or berry like with few to many arillate seeds (Te-lin et al., 1981; Joy et al., 1998 and Ke et al., 2000).

2.2. Economically important species in Zingiberaceae

Zingiberaceae consists of about 50 genera and 1300 species of pantropical origin with center of diversity in South and Southeast Asia. Some species occur in America and subtropical and warm-tropical Asia. Twenty genera, including one endemic and 216 species, including 141 endemic, four introduced, occur in China (Ke et al., 2000). A few commercially important plant species in Zingiberaceae are cardamom (*Elettaria cardamomum* Maton), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* L.), kasturi turmeric (*C. aromatica* Salisb.), mango ginger (*C. amada* Roxb.), large cardamom (*Amomum subulatum* Roxb.), *Aframomum* spp., *Kaempferia* spp. etc. Many are used in ayurvedic and other native systems of medicine from time immemorial, and some are important spices.

Skornickova *et al.* (2007a) reported the chromosomal and genome size variation in the majority of *Curcuma* species from the Indian subcontinent. They concluded that in majority of Indian taxa the basic chromosome number is x=7; with 6x, 9x, 11x, 12x and 15x ploidy level. They also reported a new species combination, *Curcuma scaposa* (Nimmo) Skornick. & M. Sabu, comb. nov.

Economically most important genera of the family are *Zingiber* and *Curcuma*. Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) are the two most economically important species in these two genera (Anonymous, 2010).
2.2.1. Ginger

Ginger of commerce is the fresh rhizomes of \textit{Zingiber officinale} Rosc. Ginger is known by various vernacular names such as Ginger in English, Adrak or Allam in Hindi, Chukku (dried ginger) or Inchi in Malayalam, Sunthi or Ardraka in Sanskrit, Inghi or Haliya in Malay, Khing in Thai, Kiang in China, Shoga in Japan etc.

Ginger is a perennial aromatic rhizomatous herb, cultivated as an annual and thought to have originated in the tropical jungles of Asia and commercially grown in India, China, South-East Asia, West Indies, Mexico, Africa, Fiji and Australia (Bone, 1997). Three races of ginger have been reported in Germany, Haliya betal (with pale colour rhizomes), Haliya bara and Haliya indang (with reddish rhizome very pungent and used in medicine). Roche and Lang (2008) reported wild ginger from Cascade Mountains.

Large ginger rhizome is known as ‘Hands’. Ginger, onion and garlic are known as ‘Trinity roots’ in Ayurveda. It is called as ‘Vishwabsheshak’ or Universal medicine in ‘Charaka Samhitha’. Ginger reduces inflammation by removing free-radicals and its ingredients are better antioxidants than vitamin C (Leonard, 2008).

The Latin term \textit{Zingiber} is derived from ancient Tamil word \textit{Ingiver} meaning ginger rhizome. There are also some thought about the origin of word ‘Zingiber’ that it is derived from Sanskrit word \textit{Singavera} meaning antler-like or horn shaped, indicating the shape of the rhizome. In ancient India, ginger was considered as Mahoushadi (great cure). In ancient Ayurvedic texts like Charaka Samhita
had prime place in curing number of ailments. The earliest report on the cultivation of ginger was in the travelogue by Tudella, who travelled between 1159 and 1173 A.D. In many other travelogues, there was mention about Calicut, Cochin, Alleppey and Quilon gingers. During 1430 A.D., the best and the most exported ginger was Malabar ginger, than that grown in any place of India. Ginger is believed to be originated from Southeast Asia. From ancient times the ginger had been cultivated in India and China. The major ginger growing countries are India, China, Jamaica, Taiwan, Sierra Leone, Nigeria, Fiji, Mauritius, Indonesia, Brazil, Costa Rica, Ghana, Japan, Malaysia, Bangladesh, Philippines, Sri Lanka, Solomon Islands, Thailand, Trinidad and Tobago, Uganda, Hawaii, Guatemala and many Pacific Ocean Islands (Ravindran and Babu, 2005).

2.2.1.1. Medicinal properties

Ginger is extensively used in various systems of medicine such as Ayurveda, Folk, Homeopathy, Siddha and Unani (Guenther et al., 1975; Weiss, 1997; Udayan and Balachandran, 2009). Fresh and dry gingers are used as a single medicine for internal use and also as an ingredient in compound medicine. It is used externally, as an adjuvant, as an antidote and also for the purification of some mineral drugs.

Ginger has various names that denote the importance or role in common life; i.e., Mahaushada- the great cure; Katubhadra- drug that has pungent taste giving goodness; Gulma moola- rhizome, which is generally spongy in nature; Anoopaja- plant that requires plenty of water for its growth; Sunti/Kaphahari/Soshana- which overcomes diseases due to kapha. In Ayurveda the properties of ginger are listed as
Rasa (taste), Katu (pungent), Guna (property), Laghu, Snigdha (light and unctuous), Veerya (potency), Ushna (hot), Vipaka (metabolic properties), Madhura (sweet) (Remadevi et al., 2005).

The medical uses of ginger were recorded in early Sanskrit and Chinese texts and ancient Greek, Roman and Arabic medicinal literature. Many scientists have reported the medicinal properties of ginger (Anonymous, 2003; Portnoi et al., 2003; Patwardhan, 2003; Lopez et al., 2005; Grzanna et al., 2005; Kim et al., 2005).

In western countries, it is usually used to treat spasmolytic and digestive problems and in Indian system of medicine, it is used to promote digestion and to treat headache and toothache and to improve circulation. In Chinese medicine, fresh ginger is used to treat vomiting, cough and common cold and dried ginger is used to treat conditions of poor appetite, vomiting, diarrhoea and pallor. It increases the digestive function by stimulating gastric secretions (except pepsin). In China, ginger is reported to treat gastric ulcers due to the presence of anti-ulcer compounds (6-gingesulphonic acid, 6-shogaol and ar-curcumene). Ginger at a concentration of 54g/day may inhibit platelet aggregation. A compound, six-shogaol of ginger is found to raise the blood pressure; its antibacterial property is also proved against G^+ve and G^-ve bacteria (Bone, 1997). Ginger is used to treat motion sickness in children (Careddu, 1999). Boone and Shields (2005) reported the safety and efficacy of the use of ginger to treat pregnancy related nausea and vomiting. Keating and Chez (2002) reported the use of ginger capsules containing 250mg of ginger for 4 days daily to prevent pregnancy related nausea. It is reported that ginger also has
radioprotective activity (Jagetia et al., 2003). Jolad et al. (2005) identified 115 compounds from dry ginger and described the antiinflammatory activity of ginger.

It is used to prevent mosquito (Africa), baldness, chest pains, bleeding, abdominal distension, vomiting with blood, poisoning from bad meet or sea food, opacity of cornea, muscle pain, stiff neck, low back pain, parasites, urinary problems, prevent scurvy (China), heart diseases, belching, diabetes, elephantiasis, compress for cramps, fatigue, hicough, thirst, edema, (India), cold hands and feets (Europe, China and India), frost bite (Europe, general), kidney problems (Europe), cholera (Electric and India), heart burn, mushroom poisoning, cool the surface in children’s disease (Electric), lung congestion with phlegm, bronchitis with copious white phlegm, cough (India and China), stomach-ache (Philippines and Samoa), increase memory, intelligence, courage, determination and mental clarity, (Medieval Europe), fever (France and India), osteoarthritis, rheumatism, chronic joint pain and tissue inflammation (India, China, Europe, Nepal and West Africa), intestinal parasites (ancient Rome), aphrodisiac (Africa, Middle-East), contraception (dried root- New Guinea), morning sickness, painful menstruation (China, Electric, Medieval Europe), scanty menses (Medieval Europe), respiratory difficulties (Samoa and west Africa) (Leonard, 2008).

Ginger is used as carminative or antiflatulent. Greek physician Gales used ginger to treat conditions caused by imbalance in the body. In China, Africa, West Indies and United States, ginger is used in traditional medicine and Greek and Romans use ginger as a spice. Dried ginger is used in the manufacture of oil, oleoresin, essence and processed meat. Ginger is also used to treat migraine (Weiss,
1997; Ganguly et al., 2003). The original use of ginger is as a spice. Spices impart flavour, pungency and colour. They also have antioxidant, antimicrobial, nutritional and medicinal properties. It imparts a ‘freshness stimuli’. In India, it is mentioned in the earliest Sanskrit literature and in China the first known record is from Confucius, ‘who was never without ginger when he ate’. It was used there to treat rheumatism, toothache and malaria. It gained popularity as spice and was well enough known to be included in most herbals from 9\textsuperscript{th} century. In 13\textsuperscript{th} and 14\textsuperscript{th} centuries, it was used along with pepper and other spices (Premavalli, 2005).

It is also used in drug interaction or as an ingredient in compound medicines. Along with Bidens pilosa, Curcuma longa, Desmodium sp., Eclipta prostrata, it is used in the treatment of impaired liver function. It is used for the treatment of bronchitis with white sputum as a mixture of Capsicum spp. and Coronopus didymus. Ginger along with Pinellia ternata rhizome or with Bambusa breviflora, is used in the treatment of bronchitis with copious phlegm. It is used with Centella asiatica or with Ginkgo biloba, Cinnamomum cassia and/or Rosmarinus officinalis to improve poor circulation and vessel problems. It is used with orange juice or with Pinellia ternata rhizome for the treatment of motion sickness, hiccup and vomiting. It is used along with Zizipus jujuba fruit or with Amomum cardamomum, Crochus sativus, Eugenia caryophyllata and Myristica fragrans for the treatment of common cold. During indigestion it is taken along with Zizipus jujuba fruit or with Amomum cardamomum, Crochus sativus, Eugenia caryophyllata and Myristica fragrans or with rocksalt and lemon juice or with Cornus florida and Hydrastis canadensis. It is used with Amomum cardamomum, Crochus sativus, Eugenia caryophyllata and
Myristica fragrans or with Allium sativum juice and honey for curing asthma. Ginger along with Aloe vera gel can cure headache or toothache. It is used along with olive oil for the treatment of dandruff. A mixture of ginger with Curcuma longa can cure chronic joint pain in the elders. It can reduce acute bacillary dysentery along with brown sugar. Dry ginger boiled with butter milk is used as antipoisonous agent. Ginger along with Berberis asiaticus, Boerhaavia diffusa, Terminalia chebula and Tinospora cordifolia can reduce amoebiasis. It is also used along with long pepper and black pepper in Ayurveda for the preparation of ‘Trikatu’ (Leonard, 2008; Remadevi et al., 2005; Sharma et al., 2008).

Minaiyan et al. (2008) reported the effect of herbal extract of ginger against acute colitis. Higher oral dose of extracts (350 and 700 mg/kg) was effective to reduce ulcer severity, area and index and mucosal inflammation severity, extent and total colitis index compared to control.

Waggans (2009) reported neuroprotective effects of the extract of ginger against Monosodium glutamate (MSG) induced neurotoxicity in male Albino rats. Elshater et al. (2009) reported the effects of daily oral administration of ginger extracts for 6 weeks on plasma glucose, lipid profile and kidney function in alloxan induced diabetic rats to the ameliorating and partly curative effects in alloxan induced diabetic rats (150mg/kg). The antimicrobial activity of ginger oils was detected against Bacillus, Candida, Trichoderma, Aspergillus, Penicillium etc. (Sasidharan and Menon, 2010). Hydro-alcoholic extracts of ginger and turmeric (1:1) were proved for their anthelmintic effects against Indian earthworms (Singh et al., 2011a).
Ginger has mild antimicrobial activities (Choubey and Patil, 2009; Pundir and Jain, 2010). Its essential oil was shown to inhibit or decrease the growth of cholera and typhoid bacteria. There are also reports on its activity against Bacillus subtilis, Escherichia coli, Mycobacterium etc. (Premavalli, 2005; Gao and Zhang, 2010). Hashim et al. (2011) reported ginger rhizome as the source of milk coagulating proteins. The anti-oxidative, anti-inflammatory, anti-obesity, larvicidal and anti-microbial properties were also reported by many researchers (Hashemi et al., 2008; Okigbo et al., 2009; Booth et al., 2010; Desai et al., 2010; Lin et al., 2010; Manda et al., 2010).

2.2.1.2. Rhizome anatomy

The transection of fresh unpeeled rhizome is almost circular or oval and about 2cm in diameter with almost regular outline. Epidermis is continuous, single layered, rectangular and ruptured in some cases. The cork only develops at the time of storage. Thickness is 480–640μm. Outer layer is 300–400μm, elongated, slightly brown and irregularly arranged. Next to this 6-12 regular rows of thin walled rectangular cells are seen. The cortex is 4mm in thickness, thin walled, large hexagonal to polygonal parenchymatous cells. The cortical cells are loaded with starch grains. These grains are large, simple and ovoid, length varying from 15-65μm. Within cortex, numerous large globules of yellowish-green coloured oil cells are seen. The outermost 3-5 rows of cortical cells are devoid of oil content. The vascular bundles are scattered, collateral, closed and greater numbers are seen in the inner cortical zone. Large bundles are enclosed within a sheath of septate fibres. Phloem is made up of small, thin walled polygonal cells with well marked sieve
tubes. Xylem contains 1-9 vessels (21-66μm in diameter). Endodermis is single layered, made up of thin walled rectangular cells, smaller than cortical cells. Cells have radial walls, slightly thickened and devoid of starch grains. Pericycle is seen in single row, made up of thin walled, tangentially elongated cells which are devoid of starch grains (Ravindran et al., 2005).

2.2.1.3. Chemistry of ginger

Zingiberene is the natural hydrocarbon isolated from oil of ginger roots and is actually a mixture of zingiberene and some bisabolene. Zingiberol isolated from ginger root oil is apparently a derivative of zingiberene or isozingiberene.

The rhizome collected from Kerala is reported to have essential oils (1-2.7%), crude fiber (4.8-9.8%) and starch (40.4-59%). High amount of volatile and non-volatile oils were reported in the rhizome from Cochin, India (2.2% volatile, 4.25% non-volatile), Nigeria (2.5% volatile, 6.5% non-volatile) and Sieraleone (1.6% volatile, 7.2% non-volatile) (Vernin and Parkanyi, 2005). Ginger contains fat, carbohydrates, various minerals, vitamins etc (Premavalli, 2005) (Table 1).
Table 1. Nutritional composition of ginger (Premavalli, 2005)

<table>
<thead>
<tr>
<th>Composition</th>
<th>USDA Hand book</th>
<th>ASTA Research Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>9.38</td>
<td>7.00</td>
</tr>
<tr>
<td>Food energy (kcal)</td>
<td>347.00</td>
<td>380.00</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.12</td>
<td>8.50</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>5.95</td>
<td>6.40</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>70.79</td>
<td>72.40</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>4.77</td>
<td>5.70</td>
</tr>
<tr>
<td>Calcium (g)</td>
<td>0.116</td>
<td>0.10</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>148.00</td>
<td>150.00</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>32.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1,342.00</td>
<td>1,400.00</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>11.52</td>
<td>11.30</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.046</td>
<td>0.05</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.185</td>
<td>0.13</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>5.155</td>
<td>1.90</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Vitamin A (Retinol Equivalents)</td>
<td>15.00</td>
<td></td>
</tr>
</tbody>
</table>

The extraction with acetone is used to collect essential oils and pungent principles and other non-volatile components of its rhizome. Using hydro-distillation techniques, from dried and fresh finger, green ginger oil containing higher amount of α-zingiberene and small amount of other sesquiterpene alcohols can be extracted. The volatile oil obtained by steam distillation from the dried ground rhizome of
ginger is light yellow liquid having the aromatic characteristic odour of ginger. The essential oil and oleoresin are used widely in the food industry, compound oils for flavouring the candy, baked products, liquors, condiments, sauces etc. Chemical constituents are α-pinene, camphene, phellandrene, cineol, methylheptenone, borneol, linalool, citral, C₁₀ and C₉ aldehydes, α- and β-zingiberene, a-curcumene, farnesene, sesquiterpene alcohol. Pungency is due to gingerol, shogaol and zingerone (Masada, 1976).

Oleoresin contains the non-volatile compounds which give pungency to the rhizome. Some essential oils and other non-volatile compounds like carbohydrates and fatty acids, ginger oils and shogaols are the medicinal components of ginger. The amount of gingerols is different in different varieties. Wayanad local, Narasapattam and Maran contain 17.7-19.25% gingerols and Nadia, Karakkal and No 646 cultivars contain 20.09-21.32%. The highest percentage of gingerol content was reported in Ernad, Chernad, Rio de Janeiro and Jamaica (24.66-26.67%). Highest oleoresin was observed in No 646 (Himachal Pradesh) - 8.59% and minimum in Waynad local (5.30%). Zingerone is colourless compound with salicylaldehyde odour. Paradol is isolated from ginger oleoresin; it is pale yellow and pungent solid (Vernin and Parkanyi, 2005).

Adulyatham and Owusu-Apenten (2005) described a method for the purification and stabilization of ginger protease (GP) or Zingibain (the meat tendering agent). They can increase the half life of sodium ascorbate stabilized (0.2% w/v) GP solution from 2.1±0.16 days at 5°C to 18 months at the same temperature. Sasidharan and Menon (2010) studied the chemical composition of
volatile oils from dried and fresh ginger, analyzed by GC and GC-MS. Zingiberene was the major compound in both ginger oils. Fresh ginger oil contained geranial (8.5%) and more oxygenated compound than dry ginger. The dry ginger oil also contains ar-curcumene (11%), β-bisabolene (7.2%), sesquiphellandrene (6.6%) and δ-cadinene (3.5%).

**Ginger oil**

Ginger oil is a pale yellow to yellow liquid with lemon odour used in perfumery. It can be isolated by steam distillation by extraction with supercritical CO₂ or after hydrodistillation or by solvent extraction of dried rhizome. It is a pale yellow to yellow coloured liquid with lemon odour. The main components of ginger oil are sesquiterpene hydrocarbons, α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, farnesene, γ-selinene, β-elexene and β-zingiberene (Vernin and Parkanyi, 2005).

Gupta *et al.* (2011) reported different essential oils such as 8-cineole (10.9%), linalool (4.8%), borneol (5.6%), α-terpineol (3.6%), neral (8.1%), geraniol (14.5%), geranial (9.5%), trans-dimethoxy citral (5.0%) and geranyl acetate (6.3%) in ginger using GC-MS. Five compounds, namely trans-linalool oxide, trans-linalool oxide acetate, (Z)-dimethoxy citral, (E)-dimethoxy citral and epi-zingiberenol were reported for the first time in the oil of ginger.

**Monoterpene hydrocarbons**

Ginger contains monoterpene hydrocarbons like α-pinene, β-pinene, myrcene, β-phellandrene, limonene, P-cymene and cumene.
Oxygenated compounds

Ginger contains oxygenated compounds like 1,8-cineole, α-borneol, linalool, neral and geranial, bornyl acetate, aliphatic aldehydes, ketones, alcohols, esters of acetic and caprilyc acid and chavicol. Neral, geranial and α-terpineol are responsible for the characteristic lemony odour of ginger (Vernin and Parkanyi, 2005).

2.2.1.4. Pharmacology

Sakr (2007) reported the ameliorative effect of ginger against liver damage induced by Mancozeb (an ethylene bis-dithiocarbamate fungicide used against a wide range of fungal diseases of field crops and fruits); it was mediated by its antioxidant activities.

Kama and Aleem (2009) reported the use of ginger for lowering the body weight and treating obesity and also in lowering the concentration of plasma lipids or treating hyperlipidemia. The combination of drugs made up of Emblica officinalis and Zingiber officinale was found to be significant in lowering the level of serum total cholesterol, serum triglycerides, serum LDL-cholesterol, serum VLDL-cholesterol and increasing the level of serum HDL-cholesterol in patients of primary hyperlipidemia.

Ginger extracts obtained using n-hexane, ethyl acetate, ethanolic soxhlet showed antibacterial activity but water extract did not show any activity (Malu et al., 2009). Stoilova et al. (2007) studied the antioxidant activity and the total phenols of ginger alcohol extract. The ginger extract inhibited the hydroxyl radicals 79.6% at 37°C and 74.8% at 80°C which showed higher antioxidant activity than quercetin.
2.2.1.5. Research and development in ginger

The research on ginger was initiated in the second half of 20th century. In India ginger research was started in 1953 at Kandaghat (Himachal Pradesh), Targaon (Maharashtra), Thodupuzha (Kerala) and Ambalavayal (Kerala). Later the research works on ginger were taken over by Indian Council of Agricultural Research (ICAR) and ginger was taken up under All India Coordinated Spices and Cashew Improvement Project. In 1975, a regional station of Central Plantation Crops Research Institute (CPCRI) was established at Calicut, Kerala, for doing research on spices, mainly ginger. In 1986, All India Coordinated Research Project on Spices was set up with head quarters at Calicut, Kerala. The regional centre was upgraded to National Research Centre for Spices in 1986 and to Indian Institute of Spices Research (IISR) in 1995 (Ravindran and Babu, 2005).

2.2.1.6. Characters of some improved varieties of ginger

In the absence of seed set; clonal selection, mutation breeding and polytploidy breeding are used for the production of elite varieties. The general objectives are high yield, quality, resistance to fungal and bacterial diseases and low fiber content. Varieties IISR Mahima, IISR Rejatha and IISR Varada were released from IISR, Calicut (Table 2).
Table 2. Some improved varieties of ginger and their salient features (Ravindran et al., 2005)

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Duration</th>
<th>Mean Yield</th>
<th>Dry recovery</th>
<th>Oil</th>
<th>Oleoresin</th>
<th>Crude Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>IISR Mahima</td>
<td>200 days</td>
<td>23.2t/ha</td>
<td>23.0%</td>
<td>1.7%</td>
<td>4.55</td>
<td>3.3%</td>
</tr>
<tr>
<td>IISR Rejatha</td>
<td>200 days</td>
<td>22.4t/ha</td>
<td>19.0%</td>
<td>2.4%</td>
<td>6.2%</td>
<td>4.05</td>
</tr>
<tr>
<td>IISR Varada</td>
<td>200 days</td>
<td>22.6t/ha</td>
<td>19.5%</td>
<td>1.7%</td>
<td>6.7%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>

2.2.1.7. Related species of economic importance

Zingiber species with medicinal properties are Z. mioga Rosc., Z. montanum (Koenig) Link ex Dietr. (= Z. cassumunar Roxb.), Z. zerumbet (L.) Smith, Z. amaricanus Bl., Z. aromaticum Val., Z. corallinum Hance etc.

2.2.1.8. Production constraints

Diseases, low productivity, prevalence of low yielding local varieties and absence of sufficient quantities of healthy planting materials etc. are the major production constraints in ginger.

2.2.1.9. Diseases of ginger

Main diseases of ginger causing production constraints are bacterial wilt (*Ralstonia solanacearum*), bacterial soft rot (*Erwinia* sp.), yellows (*Fusarium* sp.) and rhizome rot (*Fusarium oxysporum* f. sp. *Zingiberi* and *Pythium* sp.).

Soft rot is one of the most destructive diseases, first reported from Surat, India and now prevalent in India, Japan, China, Nigeria, Fiji, Taiwan, Australia, Hawaii, Sri Lanka and Korea. Plants become infected at all stages of growth, mainly on buds, roots, developing rhizomes and collar regions. Symptoms are initially seen as water soaked patches at the collar regions. These regions enlarge in size, become soft and watery and eventually rot. Sprouts turn yellow and collapse. In mature plants, the leaves start yellowing from the leaf tip and spreads downwards and finally death of whole leaves occurs. The rhizome becomes brown coloured and decomposes. Only the fibrovascular strands remain as such within the decaying mass, which imparts foul smell and attracts opportunistic fungi, bacteria and insects.

The causative organism are *Pythium aphanidermatum* (Edson) Fitz., *P. butleri* Subram., *P. complectens* Braun, *P. deliense* Meurs, *P. gracile* (de bary) Shenk, *P. graminicolum* Subram., *P. myriotylum* Drechsler etc.
The disease can be prevented by some cultural practices like healthy rhizome selection, narrow ridge cultivation, mulching with leaves of *Azadirachta indica* or *Glycosmis pentaphylla*, soil solarization etc. It can also be controlled by chemical control by soaking seed rhizome in Ceresan (0.25%), Agrosan-GN (0.25%), Dithane M-45 (Mancozeb) and other fungicides for 30 minutes, and soil drenching with Bordeaux mixture (4:4:50), Perenox (0.35%), Dithane Z-78 (0.15%), 0.1% Mercuric chloride, etc. and also controlled by biological agents using *Trichoderma lignorum*, *T. harzianum*, *Gliocladium virens* etc. (Dohroo, 2005).

Chauhan and Patel (1990) reported the use of chemicals against *Pythium* spp. and *Fusarium* spp. Among different chemicals tested, all metalaxyl formulations were effective against *Pythium* species and Bordeaux mixture (3000 ppm) gave the best result against *Fusarium solani*.

Yellow is another dangerous stem rot disease, reported for the first time from Queensland. Infected leaves become yellow coloured, and it gradually spreads from the margin to entire leaves, causing the death of whole plant. Older leaves are infected first followed by the younger ones. Plants show premature drooping, wilting, yellowing and drying and finally death. Shriveling and central rotting of rhizome and stunting of the whole plant are prominent. The basal parts become soft and watery and the shoot becomes detached from the rhizome.

It is caused by five *Fusarium* species, mainly *Fusarium oxysporum* Schlecht. The main control measures are the use of healthy rhizomes, resistant cultivars (*SG 666*, *Kerala local*), chemical control using Ceresan wet (0.5%), Dithane Z-78 (0.2%) etc. The incidence of disease can be controlled up to 76% using intercropping of
ginger with Capsicum. Biological control is reported using *T. harzianum*, *T. hamatum* and *Bacillus subtilis*. Multiplication through *in vitro* technique is also recommended for pathogen free rhizome production (Dohroo, 2005).

Leaf spot is another dangerous disease, which was firstly reported from Godavary and Malabar regions of India, but now wide spread in most of the ginger growing countries. The symptoms are observed as small, oval to elongated spots with white papery centers and dark brown margins surrounded by yellowish halos. The spots coalesce and become large, and later the leaves become shredded and disfigured.

The pathogen is *Phyllosticta zingiberi* T. S. Ramakr. Burning of diseased crop debris is one of the most important practices to control disease spreading. The disease can be controlled by using chemicals such as Bordeaux mixture, Dithane Z-78 (0.2%), Dithane M-22 (0.2%), etc. Resistant cultivars like *Narasapatom*, *Tura*, *Nadia* etc. are also used for disease control. Diseases like storage rots caused by *Fusarium oxysporum*, *Pythium deliense*, *P. myriotylum*, *Geotrichum candidum*, *Aspergillus flavus* etc. were also reported. Other minor diseases are leaf spot caused by *Leptosphaeria zingiberi*, basal rot caused by *Sclerotium rolfsii*, violet rot caused by *Helicobasidium mompa* etc. (Dohroo, 2005).

Many bacterial diseases are also seriously affecting ginger. The most destructive one is bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi (Smith). It can be controlled by selection of healthy rhizome, chemical treatments, heat treatments etc. (Kumar and Hayward, 2005).
Many viral diseases are reported in ginger like mosaic disease caused by Ginger mosaic virus; Chlorotic fleck virus, Tomato big bud organisms are another pathogens. Nematodes like *Meloidogyne incognita*, *Radopholus similis*, etc. Chirke virus and Vesicular–Arbuscular Mycorrhizal (VAM) association are other minor problems in the ginger cultivation (Dohroo, 2005).

Many insects are also seriously infesting ginger. *Odontotermes obesus* Holm., *Pentalonia nigronervosa* Coq., *Pseudococcus* sp., *Aspidiella hartii* Ckll., *Aspidiotus destructor* Sign., *Thrips tabaci* Lind. etc. are some of them (Devasahayam and Koya, 2005).

### 2.2.1.10. Common practices for prevention of disease

Hot water treatments, exposing of seed rhizomes to a constant temperature of 50°C for 10 minutes to control nematodes, use of 10% bleach solution for 10 minutes (Valenzuela, 2011) are some of the common practices of prevention of diseases.

Stirling (2004) reported the causes of poor establishment of ginger due to the pathogen *Fusarium oxisporum* f. sp. *Zingiberi* (Foz). The pathogens were isolated from fungicide treated seed pieces that were ready for planting and from newly planted seed rhizome.

Ghosh and Purkayastha (2003) reported systemic protection of ginger against pathogen *Pythium aphanidermatum* causing rhizome rot. This pathogen was detected after eight weeks of inoculation by agar gel double diffusion and immunoelectrophoretic tests and after one week of inoculation by indirect ELIZA. Systemic
protection was carried out by soaking the rhizomes in synthetic chemicals or herbal extracts for one hour prior to sowing. Among different materials tested, Jasmonic acid (5mM) and 10% *Acalypha indica* leaf extracts reduced the intensity of disease by producing defense related proteins. Atleast four defense related proteins (DRPs) were detected in the *Acalypha* treated leaves of ginger plants.

### 2.2.1.11. Micropropagation of ginger

After Hosoki and Sagawa (1977), many researchers have reported micropropagation of ginger using shoot meristem (Nadgauda *et al.*, 1980; Pillai and Kumar, 1982; Ilahi and Jabeen, 1987; Bhagyalakshmi and Singh, 1988; Saradha and Padmanabhan, 1989; Balachandran *et al.*, 1990; Choi, 1991a; Choi and Kim, 1991; Samsudeen, 1996; Babu, 1997), base of the pseudo-stem (Ikeda and Tanabe, 1989; Choi, 1991b; Babu, 1997; Babu *et al.*, 1998) and root (Nel, 1985). The most common medium used was MS basal medium supplemented with IAA, IBA, NAA, 2,4-D, Kinetin and BAP in different concentrations and combinations. Babu (1997) studied plant regeneration from other explants like vegetative buds, immature inflorescence, leaf, ovary and anther on MS basal medium supplemented with cytokinin and auxins. MS medium containing auxin (NAA) and cytokinin (BAP) gave positive responses in inducing multiple shoots and roots. Balachandran *et al.* (1990) reported an *in vitro* clonal propagation technique for ginger. Among different media combinations with BAP and Kinetin tried, 3mg/l BAP without Kinetin was found most responsive. He also reported shortterm conservation of shoot cultures in tubes with polypropylene caps upto 7 months without genetic variation.
Babu et al. (1992a) reported indirect in vitro plant regeneration from callus tissue formed on the excised tissue from young callus of ginger cv. Maran on MS medium supplemented with different concentrations of BA and 2,4-D. In MS medium supplemented with 0.9µM of 2,4-D and either 23.2µM of Kinetin or 22.2µM of BA, low rate of organogenesis was noticed. The number of shoots per culture was higher in medium with Kinetin (10-25). The developed shoots were placed in MS medium containing 5.4µM of NAA for rooting. The plantlets developed were established in the field with above 80% success.

Babu et al. (1992b) reported a direct regeneration technique of ginger from immature inflorescence (i.e., using whole inflorescence or single flowers), on MS medium supplemented with 10mg/l BA, 0.2mg/l 2,4-D and solidified with 6gl/l agar and incubated at 16-hour photoperiod. Rooting was carried out in medium with 1mg/l NAA. It was reported that more than 80% of the cultures got established in the soil.

Rout et al. (2001) reported a shoot multiplication system in ginger cv. V3S18 using meristem on MS medium supplemented with 26.6µM BA, 8.57µM IAA and 1111.1µM Adenine sulphate and 3% sucrose. These shoots developed in vitro rhizomes on MS medium supplemented with 4.44µM BA, 5.71µM IAA and 3–8% sucrose at 24h photoperiod.

Shylaja et al. (2003) reported the adventitious regeneration in ginger cultivars Maran and Reo de Janaero using MS medium supplemented with 3mg/l BA. High positive result was obtained in variety Maran. Arimura et al. (2000a) developed micropropagation technique in ginger cv. Atibaia using etiolated shoots,
which were produced under dark condition on MS medium containing different concentrations of NAA. These etiolated shoots were transferred in to MS medium supplemented with 25µM Kinetin and the regenerants produced were rooted in growth regulator free MS medium for rooting and finally established in the field condition.

Soo et al. (2000) developed an efficient culture technique for ginger. Callus was developed on N6 medium supplemented with 2mgl⁻¹ NAA. Plant regeneration occurred on MS medium supplemented with 1–2mgl⁻¹ BA. Mohammed and Quraishi (1999) reported the clonal propagation of ginger through shoot tip culture on MS medium supplemented with 2mgl⁻¹ BAP and NAA.

According to Pu et al. (2004) the optimum medium requirements for the induction of callus in ginger was MS medium supplemented with 2mgl⁻¹ 2,4-D and 1mgl⁻¹ Kinetin. Plantlet regeneration was obtained in the medium containing 2mgl⁻¹ Kinetin, 0.5mgl⁻¹ NAA. Palai et al. (2000) reported the regeneration of ginger cvs. Suprava, Turia local, Suruchi and V3S18 on MS medium supplemented with 5mgl⁻¹ BA, 1.5mgl⁻¹ IBA, 100mg⁻¹ Adenine sulphate and 3% sucrose, and rooted on half strength MS medium supplemented with 0.25–0.5mgl⁻¹ IAA or IBA. They also reported the presence of peroxidase, catalases during rooting and acid phosphatase activities during organogenesis.

Lincy et al. (2004) reported an efficient protocol for direct regeneration of two varieties of ginger, ‘Jamaica’ and ‘Australia’ through culture of basal and middle segment of aerial shoot. Medium used was MS basal medium supplemented with various concentrations of BAP and NAA. Among different explants used, 95%
of the cultures containing the basal part (axillary meristems) and 70% of cultures containing middle part of aerial stem gave good results. Among the two varieties, variety Jamaica showed good response than the other. The combinations 1:0.5, 1:1, 2:1, and 3:1 mg/l of BAP and NAA produced more good results. Eighty five percent of the cultures were established in the field and after a season of growth these plants yielded approximately 100g fresh rhizome per plant.

Hepperly et al. (2004) reported production of ginger rhizomes free from bacterial wilt using rhizome produced from tissue cultured plants under greenhouse production and management system. The plants were planted in plastic grow-bags (16×16×30 inches) filled with growing medium (containing Triple superphosphate, Gypsum, Granular fertilizer (Gaviota 10-5-40), Scott’s Micro-max Plus Amendment Mix, Maiden well All Purpose Hi-Silica Growing Media (diatomaceous earth) mixed with equal parts by volume of four slow-release fertilizers) and placed on 3×8 ft benches. Each plant was irrigated four times a day for 10 minutes per session to provide 2 gallons of water per bag per day. During harvesting rhizomes of average yield per bag was 14.9lb.

Kirdmanee et al. (2004) reported a novel technique for the production of bacteria-free ginger rhizomes through biotechnology. Initiation of the culture was conducted using buds of different sizes, cultured on MS medium supplemented with 15% coconut water for rapid screening of bacterial infection. The bacteria free shoots were cultured in MS medium supplemented with various concentrations of BA and 2-iP. The medium containing 4mg/l BA was found to be most responsive.
The lowest bacterial infection of meristem was reported in the shoots cut in to 0.15±0.05mm in diameter.

Muda et al. (2004) developed an *in vitro* propagation protocol for 3 varieties of ginger, *Zingiber officinale* var. *officinale*, *Z. officinale* var. *rubrum* (haliabara), *Z. officinale* var. *rubrum* (haliapadi). Most responsive medium was MS medium supplemented with 3% sucrose, 0.2% phytagel and 1–3mg l⁻¹ BA.

*In vitro* regeneration of ginger plants from vegetative buds in MS medium supplemented with 2mg l⁻¹ BA and 2mg l⁻¹ IBA and higher rate of establishment of tissue cultured plants in the field was reported by Keng and Hing (2004).

Plant regeneration can be induced from floral meristem from young inflorescence. These shoots grow in to complete plants within 7-8 weeks time. The development and maturity of ginger flower during *in vitro* culturing can be used to produce aseptic pollen, which can be used for the conservation in cryopollen genebank and for *in vitro* pollination (Babu et al., 2005).

Guo and Zhang (2005) reported *in vitro* regeneration from somatic embryogeneic cell suspension culture from shoot tips of four cultivars of ginger on MS medium, and four modified MS media; MSN (MS medium with half concentration of NH₄NO₃), MSK (MS medium with half concentration of KNO₃), MS/2 (MS with half strength of all components) and MSCH (MSN medium with 500mg l⁻¹ of casein hydrolysate), supplemented with 2,4-D, NAA, BA or Kinetin either alone or in combinations. Among these phytohormones, 2,4-D at a concentration of 1mg l⁻¹ was most responsive to induce embryogenic callus. The
decrease in the concentration of NH$_4^+$-N was found to increase the induction of embryogenic callus. The best medium for embryogenic callus induction was MSN containing 1mgL$^{-1}$ 2,4-D and 0.2mgL$^{-1}$ Kinetin.

Bhattacharya and Sen (2006) reported an in vitro micropropagation technique of ginger using MS medium supplemented with different concentrations and combinations of cytokinins (BAP, Zeatin and Kinetin). Maximum numbers of plantlets/explant were obtained in the medium supplemented with 4mgL$^{-1}$ BAP along with 2mgL$^{-1}$ Kinetin (8.94). But maximum plantlet height was obtained in the medium supplemented with 4mgL$^{-1}$ BAP and 3mgL$^{-1}$ Kinetin (8.38cm). The regenerated plants showed about 94-100% establishment in the field, and there was increase in the yield, about 2 fold, compared to the conventionally propagated rhizomes. Kavyashree (2009) reported an efficient protocol for in vitro multiplication of ginger through direct regeneration of vegetative buds on medium supplemented with 17.76µM BA with 96% positive response. The repeated subculture resulted in rapid shoot multiplication at the average rate of 4 fold per culture. Shoots and roots were induced at a rate of 19.1 and 12.3 respectively. The regenerated plants showed 86% survival frequency after few days of indoor acclimatization.

Ho et al. (2007) developed an in vitro multiplication technique for ginger on MS medium supplemented with 0.1mgL$^{-1}$ NAA, 1mgL$^{-1}$ Kinetin and 3% sucrose, which produced long shoots. On MS medium supplemented with 0.5mgL$^{-1}$ NAA, 2mgL$^{-1}$ BA and 3% sucrose, cultures produced more numbers of shoots. Increase in
concentration of sucrose to 6% along with 0.1mg l⁻¹ NAA and 0.5mg l⁻¹ BA enhanced both proliferation and growth of shoots without hyperhydricity.

Ahmad et al. (2007) reported an efficient multiplication protocol for ginger. Among different growth regulators used with varying concentrations, a combination of 17.6µM BA, 2.46µM IBA, 0.96µM Kinetin and/or 11.42µM IAA with ½ and ¾ strength MS media showed more growth, which also produced simultaneous rooting.

Hua et al. (2007) reported the induction of embryogenic callus from ginger shoot tips on MS medium with half concentration of NH₄NO₃ and supplemented with 1mg l⁻¹ 2,4-D and 0.2mg l⁻¹ Kinetin. The protoplasts isolated were cultured initially in liquid medium with 1mg l⁻¹ 2,4-D and 0.2mg l⁻¹ Kinetin. The protoplast derived calli produced embryos on medium containing 0.2mg l⁻¹ 2,4-D, 5mg l⁻¹ BA, 3% sucrose and 0.7% agar. The embryos developed shoots on MS medium devoid of any growth regulators. Shoots developed in to complete plantlets on MS medium supplemented with 2mg l⁻¹ BA and 0.6mg l⁻¹ NAA.

Jamil et al. (2007) developed an efficient protocol for the regeneration of ginger using shoot apical meristem. Callus was initiated on MS medium supplemented with 0.1mg l⁻¹ NAA, 1–2mg l⁻¹ Kinetin, 1mg l⁻¹ IAA and 1–2mg l⁻¹ BA. Maximum shoot differentiation from callus was reported on medium containing 0.1mg l⁻¹ IAA, 1mg l⁻¹ BAP. Plants regenerated were hardened in soilrite and then transferred to field with 90% establishment. The regenerated plants from MS medium with 0.1mg l⁻¹ NAA and 0.1mg l⁻¹ BAP were exposed to elevated CO₂ (400–4000ppm increase in atmospheric CO₂) for enhanced adventitious bud and shoot formation.
Paul *et al.* (2007) reported an *in vitro* method for plantlet regeneration in ginger using various explants like sprouted bud, shoot tip, leaf and pseudostem. Explants from cv. *Maran* and cv. *Rio-de-janeiro* were cultured on MS medium supplemented with different concentrations of 2,4-D and BA and incubated at light and dark. Shoot morphogenesis was achieved from one month old calli inoculated on MS medium supplemented with 3mg l⁻¹ BAP. Further shoot proliferation and rooting were done in the same medium. After hardening the plants were established in the field.

Suma and Keshavachandran (2007) isolated young buds of ginger and cultured on MS medium supplemented with 3mg l⁻¹ BA, which induced shoot regeneration. Among different concentrations and combinations of hormones used, 1mg l⁻¹ 2,4-D and 0.5mg l⁻¹ BA were most effective in inducing and maintaining embryonic cultures. The somatic embryos were germinated on half strength MS medium supplemented with 3% sucrose and 3mg l⁻¹ BA.

Soto *et al.* (2008) developed a low cost glass fermenting device, containing 350ml of liquid medium, which could propagate 50-75 explants during micropropagation of ginger. Jo *et al.* (2007) reported an efficient bioreactor system for the micropropagation of ginger on MS liquid medium supplemented with 0.3mg l⁻¹ NAA and 2mg l⁻¹ Kinetin. They reported that 10L bottle type bioreactor is more efficient than 250ml Erlenmeyer flasks.

Shirsat *et al.* (2008) reported micropropagation of ginger cv. *Varada* using rhizome buds on MS medium containing 2.5 ppm BAP and 0.1 ppm NAA for shooting and rooting and the regenerated plants showed 40% establishment in the
fields. Lincy and Sasikumar (2010) reported a protocol for shoot regeneration of ginger cultivars *Jamaica* and *Varada*. Maximum growth and development was observed on medium containing TDZ and IBA (1:1, 1:0.1mgL⁻¹). The hardening medium containing soil:sand:coir dust:cow dung and 5g *Trichoderma harzianum*/cup gave good results on survival, plant height, number of leaves and chlorophyll content.

Kambaska and Santilata (2009) investigated the effect of different concentrations and combinations of BAP and NAA on *in vitro* shoot and root induction in ginger cultivars *Suprava* and *Suruchi*, using fresh rhizome sprouting bud in semisolid MS media. Among different media used, more shoot multiplication was observed in the medium containing 2mgL⁻¹ BAP and 0.5mgL⁻¹ NAA (7.5±0.45 shootlets with a mean shoot length of 6.2±0.37cm per explant). *In vitro* shootlets were rooted in half strength MS basal medium supplemented with 2mgL⁻¹ NAA and about 95% cultures responded with an average number of 8.5±0.33 roots per explant with average length of 3.5±0.38cm. The survival rate of regenerated plants was about 95%. Hamill *et al.* (2009) reported the production of micropropagated ginger plants as a source of disease and pest free stock to establish a clean seed scheme based on the production of conventional planting material. Minas (2010) reported a method used for the production of pathogen free and genetically uniform ginger microplants to be used as propagation stocks and for the mass propagation of new cultivars for rapid release of growers.

Ying *et al.* (2009) conducted tissue culture of ginger seedling, to improve poor quality and yield caused by its conventional tuber and asexual reproduction.
The regenerated plants showed rapid growth, vigour, disease resistance, strong adverse resistance etc. The tubers were bright yellow in color, uniform in size and heavy peppery in taste. The plants also showed high quality and high yielding capacity (5000kg/667m²).

Behera and Sahoo (2009) reported an efficient simple micropropagation method for ginger using fresh rhizome sprouting bud in semisolid MS medium supplemented with 2mg l⁻¹ BAP and 5mg l⁻¹ NAA. Regenerated plants were rooted on half strength MS medium supplemented with 2mg l⁻¹ NAA. The hardened plants survived with 95% success in the field.

Villamor (2010) reported that the lack of healthy planting materials was the reason for decline in ginger production. In vitro studies indicated that nitrogen in the form of KNO₃ significantly improved proliferation rate of in vitro cultures of ginger in both full and half strength MS media. Leaf growth and root formation were better in media devoid of NH₄NO₃.

Hossain et al. (2010) reported a suitable protocol for plant regeneration of two exotic and six locally cultivated varieties of ginger (Fulbaria, Syedpuri, Chittagongi, Jangli, Indian, China, Sherpuri and BARI ada–1). In these studies they confirmed that variety Indian was the best for root induction and callus induction. MS medium supplemented with 4mg l⁻¹ BAP, 3mg l⁻¹ Kinetin and 1mg l⁻¹ IAA showed best response for callus induction and half strength MS supplemented with 2mg l⁻¹ of both BA and NAA was effective for regeneration.
Subudhi et al. (2010) developed an efficient protocol for the micropropagation of ginger using dormant axillary buds of unsprouted rhizomes of three cultivars (Suruchi, Surabhi and Phiringia) on MS medium supplemented with BA, IAA at varying concentrations. In vitro sprouted buds were transferred to the multiplication medium containing BA (1–6mg l⁻¹), IAA (0.1–1mg l⁻¹) and Adenine sulphate (50–100mg l⁻¹). Each variety has maximum growth in different media combinations. The regenerated plants were assessed for their drug yielding potential through evaluation of various morphological and biochemical characterization. They assumed that this method can be used for the production of disease free planting material.

Zhen-wei et al. (2010) described the internal and external research programme made and the key problems existing in ginger tissue culture. Arimura et al. (2000b) evaluated in vitro growth and development of ginger in different concentrations of NAA and BAP. BAP at 1mg l⁻¹ produced the maximum number of shoots and NAA at 0.5mg l⁻¹ promoted the number and length of roots. Liquid medium without BAP induced higher number of shoots and shoot length was influenced by NAA and BAP.

2.2.1.12. Microrhizome induction

There are many reports on the microrhizome induction in ginger, since Sharma and Singh (1995) reported the technique for the first time. Shoots were cultured in MS liquid medium supplemented with 1mg l⁻¹ BA, 2mg l⁻¹ Calcium pantothenate, 0.2mg l⁻¹ GA₃ and 0.05mg l⁻¹ NAA. After four weeks of growth, the medium was replaced by microrhizome induction medium containing MS salts
supplemented with 8mg/l BA, 75gl⁻¹ sucrose and incubated at 25±1°C under dark for microrhizome induction. Successful field establishment was also achieved.

Geetha (2002) reported multiplication and microrhizome induction in ginger. Multiplication with 90% response was achieved in the medium supplemented with 1mg/l BA and 0.5mg/l NAA. These plants were successfully established in the field with 80-100% establishment. Increased concentration of sucrose (9, 10 and 12%) induced microrhizome formation and in the field they produced rhizomes of 200-500g per plant. The efficiency of microrhizome on germplasm storage was also suggested.

Teerakathiti et al. (2003) reported the influence of light incubation and dark pre-treatment during microrhizome formation of ginger. The explants collected from in vitro multiplied stock were cultured on MS medium at 25±2°C and 60±5% relative humidity with various photoperiods for 0, 8, 12, and 16hd⁻¹. Microrhizomes cultured under dark yielded the highest dry matter during 3-weeks’ incubation and then, gradually decreased in weight and dry matter. These were produced an average number of 5.8 microrhizome/clumps with 6.78mg/microrhizome and 5.2 microrhizomes/clumps with 7.01mg/microrhizome.

Chuan-Hong et al. (2006) reported the effect of sucrose and Paclobutrazol on in vitro microrhizome formation in ginger. Among different concentrations of sucrose, Paclobutrazol and NAA used, the best result was obtained in the medium containing 0.2mg/l BA, 0.5mg/l NAA, 2.5mgl⁻¹ Ca₃(PO₄)₂ and 2.5mg/l Paclobutrazol along with 8% sucrose. The increased light also stimulated microrhizome induction.
Xue et al. (2007) reported the microrhizome induction in ginger using NAA, PP333 (Paclobutrazol), BA and sucrose and described that sucrose was the most important component in inducing microrhizome in ginger followed by PP333 and NAA. The most responsive medium was MS supplemented with 1mg l\(^{-1}\) NAA, 0.2mg l\(^{-1}\) PP333 and 8% sucrose.

Qiang et al. (2008) studied the effect of Kinetin, Gibberellic acid and NAA on microrhizome production in ginger and reported that the effect of GA was higher than the other two. The concentrations of GA, Kinetin and NAA at 1.33–2.35, 0.49–0.66 and 0.62g l\(^{-1}\) respectively produced microrhizomes of weight of over 0.25g. The optimal growth was observed in MS medium supplemented with 8g l\(^{-1}\) sucrose. MS macrosalts at double strength and normal strength microsalts with a photoperiod of 24h light made 100% establishment of the regenerants on transfer to soil.

2.2.1.13. Rhizome production from micropropagated plants of ginger

Smith and Hamill (1996) studied the growth and development of micropropagated and conventionally propagated plants in the field. In the first generation, micropropagated plants showed reduced rhizome yield and long shoots than plants derived conventionally from the seed rhizome. The plants multiplied in BAP containing medium produced larger shoots with greater leaf area than that grown in hormone free medium. Number of inflorescences was also fewer in micropropagated plants. The rhizome from conventionally propagated plants were heavier with less root mass than micropropagated plants. Plants grown in hormone containing medium grew larger with more rhizomes than the plants grown in hormone free medium. In seed derived plants, the shoot rhizome ratio was less than
one and in micropropagated plants it was greater than one. During second generation, micropropagated plants grew as same as or better than conventionally propagated plants. Plants grown in hormone containing medium grew more vigorously and produced more shoots than conventional plants. At harvest, the micropropagated plants did not show any difference in size of rhizome than control plants. Only 4% *Fusarium* yellow was seen in micropropagated plants where as in control plants it was 7%.

Freitez *et al.* (2003) reported field performance of *in vitro* grown and conventional ginger, evaluated under full sunlight and partial shade. The number of shoots, fresh weight and dry weight of shoots were higher in *in vitro* regenerated plants which were shorter. Rhizome mass was greater in conventionally propagated plants. All the variables were superior (in most cases) in partial shade independently with regard to type of propagation.

Girardi *et al.* (2007) conducted a study to evaluate the development of micropropagated ginger plantlets after 30 days of acclimatization in 3 different substrates (sand+plant max (1:1); carbonized rice shell+plant max (1:1) and sand+plant max+carbonized rice shell (1:1:1)) to obtain information on the total soluble sugar levels at different stages of plant growth. They reported the difference of field performance of micropropagated plants compared to conventionally propagated plants, i.e., they confirmed that more growth, number of leaves and proliferation were noticed in micropropagated plants. Guan *et al.* (2008) reported the different changes in the micropropagated ginger plants during acclimatization at different irradiance (60-250μmol m⁻²s⁻¹). The change in the activities of antioxidant
enzymes and photosynthetic capacity were reported. Increase in chlorophyll content and chlorophyll a/b ratio was found under irradiances. In vitro plantlets showed low photosynthesis but chloroplasts from in vitro leaves contained well developed grana and osmiophilic globules.

Lincy et al. (2008) reported the relationship between yield and yield contributing characters of micropropagated ginger plants cultivars Jamaica and Varada. This study revealed a high, positive and significant correlation of fresh rhizome yield per plant with circumference of cormlets (0.92) followed by length of cormlets (0.87) and number of cormlets (0.76). They also reported the positive effect of plant height, leaf number, leaf length, leaf width on yield of rhizome.

Girardi and Pescador (2010) reported the variation in total sugar and starch levels during the developmental stages of ginger seedlings. In the first year of cultivation, the mean starch levels were higher in micropropagated plants, both in leaves and roots. High starch levels were detected in rhizomes and buds. Based on the high survival rate of acclimatized plants and the higher levels of total soluble sugar and starch at the early stages of cultivation, acclimatization is recommended to assure higher plant survival and reserve allocation. Rahman et al. (2010) evaluated the field performance of ginger plants regenerated on MS/White rooting media with 1mgl⁻¹ IBA. Rooting performance was good in MS basal liquid rooting media with 82% of rooting. Field establishment of plant was better in soil combinations of garden soil: sand (1:1) and produced plants with an average of 11.8cm height.
2.2.1.14. Phytochemical screening

Solvent extraction with different types of solvent systems is used to obtain oleoresin extracts from ginger. Acetone or ethanol based extraction methods were developed to extract oleoresin from dried ground ginger. Ethyl ether, acetone, hexane, pentafluoropropane, hexafluoropropane etc. were also reported to obtain oleoresin (Vernin and Parkanyi, 2005).

He et al. (1998) reported a gradient elution reversed-phase HPLC separation system of ginger. The extract was prepared with 1g fresh ginger sample, refluxed with 20ml of methanol for 1h. This solution was filtered and used to run HPLC with solvents water and acetonitrile. The TLC was performed using hexane and acetone as solvents (8:2). In Korea, antioxidant components of ginger were separated by extracting with ethyl acetate after methanol extraction (Vernin and Parkanyi, 2005).

In India, more sesquiterpene compounds were isolated using HPLC technique. Using this technique ar-curcumene with more than 99% purity and α-farnesene with 84% purity can be obtained. The other sesquiterpene hydrocarbons obtained are β-sesquiphellandrene, α-zingiberene and β-bisabolene. These compounds were further purified by preparative capillary GC. The conditions used were 25×1cm column fitted with 5µM C-18 silica gel reversed phase eluted with MeCN/H₂O (88:12) with flow rate of 4ml/minute and detected at 215 and 245nm. The geranial and neral content can be measured using another system constituting 15×0.46cm fitted with microsorb 5µM C-18 silica gel and solvent system MeCN/H₂O (6/l, 1ml/minute) to MeCN/H₂O (0.5/5 in 30 minutes) and detected at 230nm. There are many reports on application of HPLC method in quality
determination of ginger due to different physiological and geographical variations. According to different extraction methods reported, the gingerol content was high when extracted with supercritical CO$_2$ (Vernin and Parkanyi, 2005).

Different chemical constituents like ar-curcumene, $\alpha$-zingiberene, $\beta$-sesquiterpene and trans $\beta$-farnesene etc. were separated from ginger using GC (Vernin and Parkanyi, 2005). The statistical analysis of GC chromatogram of ginger oil shows that four peaks of $\alpha$-terpinol, neral and geranial (peak 1), $\beta$-sesquiphellandrene and ar. Curcumene (peak 2), nerolidol (peak 3) and trans-$\beta$-sesquiphellandrol (peak 4) are accounted for 85% of the flavour response (Bednarczyk and Kramer, 1975).

Ma and Gang (2006) reported chemical analysis of ginger both conventional green house-grown or in vitro derived plants using GC/MS and LC–ESI–MS. They reported that there was no significant difference between them.

Mahdi et al. (2010) reported GC-MS fingerprinting of leaves of three micropropagated ginger cultivars namely Bukit, Tinggi Tanjung Sepat and Sabah. More than 300 compounds including aminoacids, carbohydrates and organic acids were detected and chemical variations among ginger varieties were confirmed due to genetic effects.

2.2.1.14. Molecular markers in ginger

The genetic stability of micropropagated and conventionally propagated ginger using RAPD analysis has been reported earlier (Rout et al., 1998; Mohanty et al., 2008). Rout et al. (1998) reported RAPD analysis of micropropagated and
conventionally propagated ginger. All RAPD profiles from micropropagated plants were monomorphic and similar to those of field grown control plants. No variation was detected within the micropropagated plants. Mohanty et al. (2008) evaluated the genetic stability of micropropagated clones at regular intervals of 6 months up to 24 months in culture using cytophotometric estimation of 4C nuclear DNA content and Random Amplified Polymorphic DNA (RAPD) analysis and established uniformity in fifty regenerants of ginger cv. Suprava. Geetha (2002) studied RAPD fingerprinting of in vitro conserved ginger using 10 operon random primers to establish the genetic fidelity of conserved lines. Rajalakshmi et al. (2008) reported the molecular characterization of Ralstonia solanacearum isolates using RAPD analysis.

Ghosh and Mandi (2011) reported genotype characterization and assessment of genetic diversity of fifteen wild landraces of Z. officinale Roscoe collected from different parts of northern West Bengal, India done using 16 random decamer primers. Out of 117 amplified products, 97 bands showed polymorphism (82.90%) and an average of 7.5 bands was amplified per primer. The genetic similarity coefficients among accessions were ranged from 0.673 to 0.912 (an average of 0.792).

2.2.1.16. Pathological screening

*In vitro* screening of ginger against Pythium aphanidermatum by dual culture and cell free culture filtrate studies against Aspergillus niger, A. fumigates, A. flavus and Trichoderma viride were conducted by Shanmugam and Varma (1999). Among
different fungicides (Methoxy mercuric chloride, Copper oxichloride, Mancozeb and Bordeaux mixture) tested, Mancozeb was compatible with all antagonists.

Hayden et al. (2007) reported production of high quality, pathogen/pesticide free rhizomes of ginger through aeroponic cultivation. The unique growing units were incorporated a “rhizome compartment” separated and elevated above an aeroponic spray chamber. Bottom heat was supplied to half of the plants, which accelerated the growth. One third of the plants were grown in rhizome compartment filled with perlite, other one was filled with sphagnum moss and the third one was without any aggregate medium. The plants grown in perlite matured faster than the other treatments.

2.2.2. Turmeric

Turmeric of commerce is the dried rhizomes of *Curcuma longa* L. (syn. *C. domestica* Val.) and is an important spice in India, South East Asia and Indonesia and is indispensable in the preparation of curry powders. Turmeric is known in various vernacular names like Halda or Haldi (Hindi); Manjal (Malayalam); Haridra (Sanskrit); Manjal (Tamil) etc. Turmeric, ‘the golden spice’ is the native of the Southeast Asia. The name of turmeric was originated from Medieval Latin name ‘*terramerita*’, which became ‘*terremerite*’ of French, meaning deserved earth or meritorious earth. The generic epithet ‘*Curcuma*’ is derived from the Arabic word ‘*Karkum*’ meaning yellow. It is cultivated extensively in India, Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Philippines, Africa, America and Pacific Ocean islands. India is the largest producer, consumer and exporter of turmeric (82% of the world production and 45% of the export). The area of production of
turmeric in India occupies about 6% of the total area under spice. In India, the main
turmeric producing states are Andhra Pradesh (constitutes 38% of total area of
cultivation and 45% of total production), Tamil Nadu, Orissa, Karnataka, West
Bengal, Maharashtra, Meghalaya and Kerala. In India, 70-75% of area of production
is occupied by local varieties. India exports turmeric as whole dried turmeric,
powder, oleoresin, oil and masala ingredients (Ravindran, 2007; Valsala and Peter,
2007).

Turmeric has strong association with socio-cultural life of Indian
subcontinent. It was described as ‘herb of sun’ by people of Vedic period. Ancient
Indians had given many names for it which denotes its particular properties, *Ranjani*
(Which gives color), *Mangal prabha* (bringing luck), *Krimighni* (killing worms),
*Shobhna* (indicating brilliant color), *Mahaghni* (indicating anti diabetic properties),
*Anestha* (not offered for sacrifice or homa), *Haridra* (which is dear to Lord Hari),
*Varna datri* (which gives color), *Hemaragi* (Having golden color), *Bhadra* (denotes
lucky or auspicious), *Pavitra* (holy), *Hridayavilasini* (which gives delight to heart or
charming) etc. (Ravindran, 2007).

Turmeric has atleast 6000 years of documented history of its use and socio-
cultural practices. In Atharvaveda, turmeric is prescribed to charm away jaundice,
which is the earliest reference. Reference to turmeric has also been made in
Yajnavalkyasamhitha, at the time Ramayana. The Hindus consider turmeric as
sacred and auspicious, and it is associated with several rituals, Hindu customs and
traditions, wedding ceremonies and many after-death rites since time immemorial.
In some communities the association with turmeric starts at birth and continues
towards and beyond death. In many communities, turmeric powder and rhizome are worshiped as God (Haridra Ganesha) or Goddess (Gowrie). Various Homa, Pooja, tantric designs (Kalams) etc. are performed with turmeric powder, rice powder etc. (Ravindran, 2007). Turmeric is used in various systems of medicine such as Ayurveda, Folk, Homeopathy, Modern, Siddha, Tibetan and Unani (Guenther et al., 1975; Mark, 1996; Udayan and Balachandran, 2009).

2.2.2.1. Morphology

Turmeric is a tall perennial rhizomatous herb, usually cultivated as an annual. The pseudostem is tall, robust with oblong/elliptic leaves narrowed at the base. Leafy pseudostem grows up to 1m and leaf blade having over 50cm, usually 30×7–8cm, whole green; petiole thin, broadened at the sheath; ligule lobes small (1mm); inflorescence is apical on leafy shoot, 10–15cm long, 5–7cm wide; coma bracts white or white streaked with green with light green bracts lower down; bracts adnate for less than half their length, elliptic, lanceolate, acute, length 5–6cm; bracteoles up to 3.5cm long; flowers 5–5.5cm long, petals white, staminodes and lip creamy - white with yellow median band on the lip; calyx truncate, 1cm long, minutely pubescent; corolla tube 2.5cm long, white, glabrous, lobes unequal, dorsal lobe larger, 1.5×1.7cm, concave, white hooded, hood hairy, lateral lobes linear, 1.5×1.2cm, white, glabrous; labellum 2.2×2.5cm, trilobed, middle lobe emarginated; lateral staminodes linear, 1.5×0.8cm, tip slightly curved, included within the dorsal corolla lobe; style long, filiform, stigma bilipped; epigynous glands 2-5mm long; filaments united to anther at the middle of the pollen sacs, spurs very large, broad, diverging, slightly curved with the thin apex always recurved outwards; ovary 5mm,
tricarpellary, syncarpous; ovules many with axial placentation, pubescent towards the tip, fruiting absent or extremely rare (Ravindran et al., 2007). Primary rhizome is ellipsoid with numerous emitting rhizomes of 5–8cm long, 1.5cm thick, straight or a little curved. These rhizomes bear secondary branches; deep orange colour inside and yellowish orange outside the rhizome; root tubers are rarely seen.

2.2.2.2. Floral biology and seed set

Flowering characters in turmeric vary according to the cultivars and climatic conditions. Normally flowering takes place between 109–155 days of planting. The inflorescence takes 7–11 days for blossoming after emergence of the inflorescence. Flowering period is September- December and the opening of flowers will take place between 6–6.30am. at Kerala. Anthesis is between 7–9am. Pollen grains are ovoid- spherical, light yellow and slightly sticky. Pollen stainability is 71–84.46% (Ravindran et al., 2007).

The fruit is thick walled, trilocular capsule with numerous arillate seeds. The seeds have 2 seed coats, outer thick and inner thin. Endosperm is massive and the embryo is towards the upper side of the ovule. Seeds start to germinate after 17–26 days of sowing (Ravindran et al., 2007).

2.2.2.3. Rhizome anatomy

The transections of rhizomes show distinct inner and outer zones separated by intermediate layers. Epidermis is single layered and it is followed by 2–4 layered periderm. Small outer zone contains 70–80 primary vascular bundles evenly distributed along with starch, oil cells and curcumin cells. Endodermis is continuous.
Inner zone is very large having secondary vascular bundles and high starch depositions. Xylem is composed of tracheids and vessels having helical and spiral thickening and fibers are absent. Phloem composed of sieve tubes, 1–2 companion cells and phloem parenchyma. Curcumin content and oil cells are high. Starch grains are triangular in shape and numerous in inner and outer cores. The number of starch grains varies from 12–20/cell (Ravindran et al., 2007).

2.2.2.4. Chemistry of turmeric

The genus *Curcuma* constitutes different types of secondary metabolites, including diphenylheptanoids, monoterpenes, sesquiterpenes, etc. among these curcumin and its structural analogues are most important compounds. It also contains gum, starch, minerals, yellow colorant, other nutritional components etc. (Table 3).

**Table 3. Nutritional composition of turmeric (Premavalli, 2007)**

<table>
<thead>
<tr>
<th>Composition</th>
<th>USDA Handbook</th>
<th>ASTA Research Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>11.36</td>
<td>6.00</td>
</tr>
<tr>
<td>Food energy (kcal)</td>
<td>354.00</td>
<td>390.00</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>7.83</td>
<td>8.50</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>9.88</td>
<td>8.90</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>64.93</td>
<td>69.90</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>6.02</td>
<td>6.80</td>
</tr>
<tr>
<td>Calcium (g)</td>
<td>0.182</td>
<td>0.20</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>268.00</td>
<td>260.00</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>38.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2525.00</td>
<td>2500.00</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>41.42</td>
<td>47.50</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.152</td>
<td>0.09</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Amount 1</td>
<td>Amount 2</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.233</td>
<td>0.190</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>5.140</td>
<td>4.80</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>25.85</td>
<td>50.00</td>
</tr>
<tr>
<td>Vitamin A (Retinol Equivalents)</td>
<td>Trace</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

The primary bioactive constituents in turmeric have been found to be the phenolic curcuminoids. Turmerone and ar-turmerone are the main fractions of the volatile oil derived from the rhizomes of *Curcuma*, and consist of monocyclic hydro-aromatic ketone $C_{15}H_{22}O$, *viz.* turmerone and aromatic ketone.

**Properties of Curcumin**

Curcumin is chemically 1,6-heptadiene-3,5-dione-1,7-bis-(4-hydroxy-3-methoxy phenyl)-(1E,6E) or diferuloylmethane and was first isolated in 1815, in crystalline form in 1870. The rhizome contains curcumin (curcumin I) along with other curcuminoids. The main curcuminoids are demethoxy curcumin (curcumin II), bisdemethoxy curcumin (curcumin III) and cyclocurcumin. Curcumin has antiangiogenic, antioxidant, chemotherapeutic, immunosuppressive, wound healing, anti-inflammatory, cardiotoxicity properties. It reduces cataract formation, liver injury, inflammatory bowel diseases, arthritis, lung fibrosis, gallstone formation, diabetes, multiple sclerosis, Alzheimer’s disease etc. It inhibits vascular smooth muscle cell proliferation, septic shock etc. It stimulates muscle regeneration (Ravindran, 2007). Sneharani *et al.* (2011) reported the use of curcumin as the carrier molecules in biochemical reactions.
2.2.2.5. Uses of turmeric

Varying uses of turmeric as spice and ingredient in curry powder and food preservative are described by Mark (1996). Turmeric is famed as the ‘yellow wonder’ (Chomchalow, 1996). Turmeric is extensively used as a spice, food preservative and colouring material in India, China and South East Asia (Ravindran, 2007).

Medicinal properties

Turmeric is used as stimulant, tonic, carminative, laxative, astringent and diuretic (Kirtikar and Basu, 1987). Turmeric and its components have been also assigned anti-inflammatory, analgesic, antidiabetic, anthelmintic, ophthalmic, immunoprotective and many other properties (Srimal and Dhawan, 1973; Sharma et al., 2005). It is a medicinal plant extensively used in Ayurveda and Unani medicines as remedy for various diseases from time immemorial (Remadevi et al., 2007). The authors provide a comprehensive review of the use of turmeric in various traditional medicines.

Uses as spice and colourant

Turmeric is mainly known for its yellow-orange colouring power and a musky flavour and aroma, and hence it is categorized under spices (Purseglove et al., 1981). Turmeric has been used in Asian cookery, fabric dying and cosmetics for more than 2000 years. Turmeric is valued for its characteristic colour and flavour. Essential oil, oleoresin and curcumin are the three major types of turmeric products. Distinction between these three products lies in the ratio of colour to flavour. The
principal component of colour is curcumin. Turmeric as a spice not only imparts colour to food but also enriches the flavour. The pleasant flavour of turmeric is due to the volatile oil fraction. In south India, turmeric is added to about every dish and it is an essential ingredient in all curry masala mixtures. Premavalli (2007) reviewed the use of turmeric as a spice and flavourant, citing major chemical constituents, uses of turmeric extracts, turmeric products, its preservative action, commercial use, health benefits etc.

### 2.2.2.6. Pharmacology and biological activity of turmeric and its components

Turmeric and its components are reported to have innumerable pharmacological and biological activities (Chattopadhyay *et al*., 2004; Sarker and Naher, 2007). Turmeric and its active component curcumin are used to treat atherosclerosis (Quiles *et al*., 2002; Mesa *et al*., 2003) and cancer (Baatout *et al*., 2004; Kwon and Magnuson, 2009; Yallappu *et al*., 2010; Wilken *et al*., 2011). Curcumin has anti-inflammatory (Wang, *et al*., 2008; Jurenka, 2009; Basnet and Basnet 2011), antimicrobial (De *et al*., 2009; Naz *et al*., 2010; Saleem *et al*., 2011; Mariselvam *et al*., 2012, Singh and Jain, 2012), antioxidant (Cohly *et al*., 2003; Padmawar and Bhadoriya 2011; Angel *et al*., 2013), analgesic (Singh *et al*., 2011b; Han *et al*., 2012), antiallergic (Lee *et al*., 2008; Choi *et al*., 2010; Chung *et al*., 2012) and wound healing properties (Mark 1996; Biswas *et al*., 2005; Das, 2011; Sundarananthavalli *et al*., 2011). Use of turmeric to treat knee osteoarthritis (Badria *et al*., 2002), irritable bowel syndrome (Bundy *et al*., 2004) and Alzheimer’s disease (Abascal and Yarnell, 2004) was also reported. It is also used to treat liver diseases, eye diseases, cancer and tumors, anorexia, cough, diabetic wounds, hepatic
disorders, rheumatism and sinusitis. It has hypercholesterolemic, neuroprotective, immunomodulatory, anti-HIV, nematicidal and mosquitocidal and pesticidal activities (Sarker and Nahar, 2007; Ashouri et al., 2010). It can also cure smallpox and chickenpox lesions, jaundice, leprosy etc. It has anti carcinogenic, antimutagenic, anticoagulant, antifertility, antiprotozoal, antiviral, antivenom, antiulcer, hypocholestremic, and hypotensive properties. It is also used to reduce post-operative inflammations (Valsala and Peter, 2007).

Turmeric stimulates the human immune system and destroys the brain blocking harmful proteins. It also increases the activity of intestinal flora and improves digestion. It warms, cleanses the blood and induces the formation of new blood cells (Yezovit, 2011). The immunomodulatory activity of turmeric was also reported by Jantan et al. (2011). It was also recommended to treat diseases of microbial and non-microbial origin (Hashemi et al., 2008) and obesity (Honda et al., 2006; El-Sweedy et al., 2007; Archana et al., 2010; Booth et al., 2010).

Cousins et al. (2007) studied the effects of antioxidant potential of methanolic extracts from rhizomes of four clones of turmeric grown in vitro under controlled conditions using DPPH radicals and chelate ferrous iron. They reported that commercial drying technique made negative effect of the antioxidant property of turmeric which may be reduced by using genotypic selection.

The extract of turmeric was evaluated for its analgesic activity using different animal models of analgesia. All the extracts significantly reduced the number of writhes. Turmeric extracts and its ingredients such as curcumin and bisdemethoxy curcumin are known for their therapeutic activities. In this study, a
low cost synthetic pathway has been proposed inorder to obtain glucosyl curcuminoids as water soluble potential drugs. The complete 1H and 13C NMR characterization of naturally occurring curcumin, bisdemethoxy curcumin and new synthetic glucosyl-curcuminoids were reported (Saladini et al., 2009).

Shu et al. (2009) reported the prophylactic effects and the mechanisms of curcumin on liver fibrosis in rats. The prevention of liver fibrosis may be due to the inhibition of hepatic stellate cells and induction of their apoptosis.

The effect of turmeric powder as an antifungal agent in plant tissue culture was investigated and the study revealed that turmeric powder used at the concentrations of 0.8gl⁻¹ and 1.0gl⁻¹ in the media resulted in appreciable control of fungal contamination (Upendra et al., 2011).

2.2.2.7. Research and development in turmeric

In India, the first research on turmeric was conducted at Udayagiri, Orissa in 1944 under the Imperial Council for Agriculture Research. In 1953, under the recommendation of the Spices Enquiry Committee, turmeric research was started in Kandaghat (Punjab), Targaon (Maharashtra), Thodupuzha and Ambalavayal (both in Kerala). In 1955 another research programme was started at Peddapalem (Andhra Pradesh). In 1975, all these works were coordinated under All India Coordinated Spices and Cashew Improvement Project and the research works were started at Tamil Nadu Agriculture University, Orissa University of Agriculture and Technology and High Altitude Research Station. The researches on turmeric were also taken up by Central Plantation Crops Research Institute (CPCRI), Kasaragod
and Central Food Technology Research Institute (CFTRI), Mysore. The regional station of CPCRI at Calicut, Kerala was approved as National Research Centre for Spices in 1986 and it became Indian Institute of Spices Research in 1995. In these centres various works on turmeric is going on (Ravindran, 2007).

2.2.2.8. Cultivar diversity

Variations between cultivars are seen in floral characters, aerial morphology, rhizome morphology and chemical constituents. Most wanted turmeric types are Alleppey, Madras and West Indian turmeric. United States have special preference to Alleppey turmeric (which has 6.5% curcumin content) and United Kingdom prefers Madras turmeric (which has 3.5% curcumin content). Other main turmeric cultivars are Erode and Salem turmeric (from Tamil Nadu), Duggirala, Nizamabad and Cudappah (from Andhra Pradesh), Rajpore and Sangli turmeric (from Maharashtra) (Ravindran, 2007).

2.2.2.9. Cultivars and improved varieties of turmeric

A number of cultivars are available in the country and are known mostly by the name of locality where they are cultivated. Some of the popular cultivars are Duggirala, Tekkurpet, Sugandham, Amalapuram, Erode local, Alleppey, Moovattupuzha, and Lakadong. Some of the improved varieties of turmeric and their salient features are described (Table 4).
Table 4. Some of the improved varieties of turmeric and their salient features
(Ravindran et al., 2007)

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Duration</th>
<th>Mean Yield</th>
<th>Dry recovery</th>
<th>Curcumin</th>
<th>Oleoresin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IISR Alleppey</td>
<td>210 days</td>
<td>35.40t/ha</td>
<td>19.0%</td>
<td>5.55%</td>
<td>16.0%</td>
</tr>
<tr>
<td>Supreme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IISR Prabha</td>
<td>205 days</td>
<td>37.47t/ha</td>
<td>19.5%</td>
<td>6.50%</td>
<td>15.0%</td>
</tr>
<tr>
<td>IISR Kedarum</td>
<td>210 days</td>
<td>35.50t/ha</td>
<td>18.9%</td>
<td>5.90%</td>
<td>13.6%</td>
</tr>
<tr>
<td>IISR Prathibha</td>
<td>225 days</td>
<td>39.12t/ha</td>
<td>18.5%</td>
<td>6.52%</td>
<td>16.2%</td>
</tr>
<tr>
<td>IISR Sudarshana</td>
<td>190 days</td>
<td>28.80t/ha</td>
<td>20.6%</td>
<td>7.90%</td>
<td>15.0%</td>
</tr>
<tr>
<td>IISR Suguna</td>
<td>190 days</td>
<td>29.30t/ha</td>
<td>20.4%</td>
<td>4.90%</td>
<td>13.5%</td>
</tr>
<tr>
<td>IISR Suvarna</td>
<td>200 days</td>
<td>17.40t/ha</td>
<td>26.0%</td>
<td>4.00%</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

2.2.2.10. Related species of economic importance

Other *Curcuma* species with medicinal properties are *C. amada* Roxb. (Mango ginger), *C. angustifolia* Roxb. (Wild or Indian arrow root), *C. aromatica* Salisb. (Vanharida), *C. caesia* Roxb. (Black turmeric or Black zedoary), *C. zedoaria* (Christm.) Rosc. (The long and round zedoary), *C. xanthorrhiza* Roxb. etc. (Ravindran, 2007; Skornickova et al., 2007b).

2.2.2.11. Production constraints

Low productivity, prevalence of low yielding local varieties, absence of sufficient quantities of planting materials, high cost of production in rain-fed regions, failure of rain and lack of scientific knowledge among farmers are some production constraints. Low productivity due to the lack of improved varieties and application of low levels of fertilizers and micronutrients etc. are some other factors which negatively influence the production of turmeric. Poor return due to the non-adoption of recommended post harvest processing by the farmers is another important factor that contributes to production constraints. The market price of turmeric is fluctuating on the basis of several factors including pigment (curcumin) content, the organoleptic character, the general appearance, size, physical form of the rhizome etc. Good rhizomes may have deep yellow colour, low bitter principle content, rough, hard and brittle nature with numerous encircling and ridge like annulations (Madan, 2007).
2.2.2.12. Diseases of turmeric

Diseases are the main constraints in the productivity of turmeric. Among different diseases affecting, rhizome rot and foliar diseases are most destructive in all turmeric growing regions.

Rhizome rot is first reported from Ceylon and in India it is from Andhra Pradesh and Tamil Nadu. The symptoms include progressive yellowing of leaves, which later dry up. The base of the aerial shoot shows water soaked soft lesions. The root system is also seriously affected with only brown decaying roots remaining. As the disease progresses the infection passes to the rhizome. Later this rhizome becomes soft, brown coloured and emits foul smell. Causal organism is various species of *Pythium*, *P. aphanidermatum* and *P. graminicolum* are more prominent groups. *Fusarium solani* also causes rhizome rot. These pathogens are soil borne, widely distributed with facultative mode of transmission. It thrives at a pH of 3–9 and oospore production is higher between pH 6–9.

Use of healthy rhizome, phytosanitation, crop rotation, use of resistant varieties (*Suvarna*, *Suguna*, *Sudarsana* etc.) or use of chemicals like Ridomil (0.25%), Carbendazim, Bordeaux mixture etc. are recommended for disease control (Dohroo, 2007). Leaf blotch is first reported from Gujarat (India), Saharanpur (India), and Rangpur (Bangladesh). The disease appears as yellow leaf spots of about 1–2mm diameter on both surfaces of leaves. The infected leaves become distorted with reddish brown appearance. The pathogen is *Taphrina maculans*. The primary infection occurs at the lower leaves during October to November at 80% relative humidity and 21–23°C.
The main control for the disease is the use of resistant varieties like CLL 324, Amalapuram, Mydukur, Karhadi local, CLL 326, Ochira 24, PTS 62, Acc. 360, Roma, BSR-1, JTS-1 etc. Chemical control is also recommended using Dithane Z-78 (0.2%), Dithane M-45, Blitox-50, Bavistin, Cuman L., Bordeaux mixture, Aureofungin (2.5g/l), Zineb etc. (Dohroo, 2007). The ad-hoc package of practices recommendations for organic farming from Kerala Agricultural University suggests certain control measures for rhizome rot, wilt and leaf spot diseases. Dipping the seed rhizome in 5% suspension of Pseudomonas fluorescens P1 for 15 minutes before planting, treating the seed rhizome with AMF (Arbuscular Mycorrhizal Fungi), application of organic manure enriched with Trichoderma at the time of planting, spraying and drenching the plant with Pseudomonas fluorescens P1/PGPR mix II after 45 days of planting (onset of monsoon) and at monthly intervals based on disease incidence and intensity are some of the measures suggested (Kerala Agricultural University, 2009).

Leaf spot is first reported from Tamil Nadu and severe in Cuddapah, Kurnool, Guntur, Krishna and Godavari districts of Andhra Pradesh. The symptoms are produced in the form of elliptic or oblong spots of variable size during August to September when there is high humidity in the atmosphere. The spots become coalesce and later dries up. The disease is caused by Colletotricum capsici. Its dissemination is during wet weather.

The first method of disease management is sanitation. Resistant varieties can also be used to control the disease incidence. Cultivars Nallakatla, Sugandham, Duvvur, Gandokota, P 73, Bhendi, Gadhavi, Krishna, PCT 8, PCT 10, Suguna,
Sudarshana, TC- 17, CL 520, Roma, BSR I Armoor, PTS 43 etc. are found to be resistant against leaf spot disease. Chemical control can be done by using 1% Bordeaux mixture, Dithane Z- 78, Captan, Carbendazim, Indofil M- 45, Bavistin etc. (Dohroo, 2007).

The symptoms of leaf blast are seen as dark spots on the older leaves, leaf sheaths and petals. The pathogen is *Pyricularia curcuma*. Disease management is through resistant varieties like *Sugandham* and *Rajpuri* (Dohroo, 2007).

The associated pathogens of storage rot are *Aspergillus niger*, *Macrophomina phaseolina*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Sclerotium rolfsii* etc. Storage rot caused by *Aspergillus niger* can be controlled by pre and post inoculation treatment with Bavistin and Benomyl at 10ppm. Infection caused by *Sclerotium rolfsii* can be controlled by treating the rhizome with Ceresan wet. Hot water treatment of seed rhizomes can also control the pathogen growth (Dohroo, 2007).

Nematode infestation is mainly reported in Andhra Pradesh, Kerala and Tamil Nadu. The pathogens causing crop loss are *Meloidogyne* sp., *Rhadopholus similis*, *Rotylenchulus* sp., *Hoplolamus* sp., *Criconemoides* sp., *Longidoros* sp. and *Pratylenchus* sp.

Five biocontrol agents *Verticillium chlamydosporium*, *Paecilomyces lilacinus*, *Fusarium* sp., *Aspergillus nidulans* and *Scopulariopsis* sp. which suppress root knot nematode populations. Resistant genotypes are also recommended against
its infestation. Soil application of Aldicard at 1kg/ha during third and fifth month of planting and/or 10-15kg/ha at the time of field preparation is also recommended followed by irrigation (Dohroo, 2007).

Another major threat is the attack of insect pests. Over 70 species of insects are reported to infect turmeric at the field and storage. Most serious one is shoot borer (*Conogethes punctiferalis* Guen.) which is widely distributed in India. Its larvae feed on the tender growing parts of shoot resulting in yellowing and drying of the shoot. Presence of bore holes in the pseudostem is the symptom of its attack. The crop loss due to shoot borer can be reduced by using tolerant variety like *Mannathy local* or by the use of natural enemies like *Dolichurus* sp., *Xanthopimpla* sp., *Phanerotoma hendecasisella*, *Temeluch* sp., etc. Biopesticides like Bioasp and Dipel produced from *Bacillus thuringiensis* along with Malathion are used against shoot borer. Spraying of Dipel 0.3% is most suitable. Neem oil 1% or commercial neem products (1%) are also used in the management of shoot borer. Sex pheromones are used in China, Japan, Korea and India (Devasahayam and Koya, 2007).

Rhizome scale (*Aspidiella hartii* Ckll.) is another threat. It is mainly reported from tropical regions of Asia, Africa, Central America and Caribbean Islands. It infests the rhizomes at the field and storage. In the field the pathogen attacks the plant at its early stages and later the plant withers and dries. During storage, pest’s infestation results in the shriveling of buds and rhizomes. The use of natural enemies like *Physcus* sp., *Adelencyrtus* sp. etc. is reported against shoot borer. Use of Quinalphos is also recommended against it (Devasahayam and Koya, 2007).
2.2.2.13. Micropropagation in turmeric

Turmeric is conventionally propagated through rhizome bits having one or two viable buds. High morphological variations are observed among the cultivated types. Somaclonal variation, protoplast fusion, recombinant DNA technology etc. have been made use for the improvement, conservation and utilization of the diversity and high productivity of this plant.

Micropropagation of turmeric was reported for the first time by Nadgauda et al. (1978). They cultured young vegetative buds excised from cultivars Duggirala and Tekkurpeta on MS basal medium supplemented with coconut milk, Kinetin and BA or on Smith’s medium supplemented with coconut milk, Kinetin, BA and Inositol. The plantlets were subcultured on the same medium.

Followed by this, Sato et al. (1987), Yasuda et al. (1988), Balachandran et al. (1990), Sugaya (1992), etc. published their work on in vitro regeneration in turmeric. Among these, report of Balachandran et al. (1990) on in vitro clonal propagation technique for turmeric was widely discussed. They used different concentrations of BAP and Kinetin and 3mg l\(^{-1}\) BAP was selected as the most responsive concentration. They also reported short-term conservation of cultures in tubes with polypropylene caps upto 7 months without genetic variation.

There are many reports on organogenesis and plantlet formation from callus cultures of turmeric. Shetty et al. (1982) reported callus regeneration of turmeric on modified MS medium containing 40g l\(^{-1}\) sucrose and 0.5mg l\(^{-1}\) Kinetin. During subculturing and exposing to light, the callus produced several buds that turned
green and developed plantlets. Salvi et al. (2001) reported callus induction and plant regeneration of turmeric from leaf base on MS medium supplemented with Picloram, NAA and BA. Best result was obtained when used 2mgL⁻¹ Picloram, 5mgL⁻¹ NAA and 0.5mgL⁻¹ BA in combination with 0.1mgL⁻¹ TIBA or 2,4-D. The green shoot primordials developed from the callus were transferred to regenerating medium containing Kinetin, the rooting was done in hormone free medium. The RAPD analysis of cultures using 14 primers showed 38 novel bands and about 51 bands present in mother plant were absent in the regenerants. This indicates the genetic variability during in vitro culture.

Nazeem and Menon (1994) reported seed set in controlled crosses of short duration turmeric types. Sajina et al. (1997) reported development of synthetic seeds in turmeric by encapsulating the somatic embryos and shoot buds in calcium alginate. These synthetic seeds were viable up to 7 months.

Salvi et al. (2000) reported direct shoot regeneration from immature inflorescence of turmeric cv. Elite on MS medium supplemented with IAA and BA/NAA, TDZ and IAA. Regenerated shoots were grown on MS medium for further development and later transferred to medium supplemented with 0.1mgL⁻¹ NAA for the induction of roots. Renjith et al. (2001) reported in vitro pollination and hybridization between 2 short duration types, VK- 70 and VK- 76 to develop high yielding variety with high curcumin content. Seed set as well as seed development was obtained by in vitro stigmatic pollination. The shoot multiplication was attained on ½ strength MS medium supplemented with 3% sucrose, BA and NAA. This reduced the breeding time and helped in recombination breeding.
Salvi et al. (2002) reported a protocol for *in vitro* propagation of turmeric cv. *Elite*, using young vegetative buds from sprouting rhizomes. According to them, liquid medium was more favourable than solid medium for shoot multiplication. Among various carbon sources used, sucrose, fructose, glucose, market sugar etc. were found to be equally effective and xylose, rhamnose, lactose and soluble starch were inhibitory. The medium supplemented with Kinetin riboside, BA riboside, 2i-P and metatopolin N\(^6\)-(hydroxybenzyl) adenine produced highest average number of shoots.

Lakshmi and Mythili (2003) reported development of synthetic seeds by encapsulating somatic embryos in calcium alginate beads. Zapata et al. (2003) developed a methodology for *in vitro* propagation of *C. longa* through indirect organogenesis. Vegetative shoot tips were grown in MS medium supplemented with NAA and BA, the vigorously grown plantlets were selected for the induction of callus in medium containing TDZ (1.5mg\(l^{-1}\)) and BA (0.2mg\(l^{-1}\)). The induction of shoots was obtained in the medium containing NAA and BA. For acclimatization, a hydroponic system was used, which helped the plants for the adaptation to lower relative humidity.

Prathanturarug et al. (2003) studied the effect of plant growth regulators, explants types and culture regimes on *in vitro* shoot regeneration from terminal bud explants of *C. longa*. Maximum shoot induction was noticed in medium containing 18.17\(\mu\)M TDZ with divided and undivided explants. With the exposure of the explants to a high concentration of cytokinin prior to transfer to plant growth regulator free MS medium, they could obtain increased shoot proliferation and
rooting. The regenerated plants were then successfully established in the greenhouse and subsequently under field conditions without any variation from their parent plants.

Roy and Raychaudhuri (2004) reported a protocol for in vitro plantlet regeneration in four species of *Curcuma* and curcumin content estimation in regenerants. *In vitro* regeneration was carried out from nodal explants (*C. longa*, *C. amada* and *C. aromatica*) and rhizome explants (*C. zedoaria* and *C. amada*). *C. longa* and *C. aromatica* regenerants showed high curcumin content.

Rahman *et al.* (2004) reported an *in vitro* regeneration technique for turmeric using MS medium supplemented with different concentrations and combinations of cytokinin and auxin. The best shooting response was obtained in the medium containing 2mg/l of BA. Rooting trials carried out using half strength MS medium supplemented with different concentrations of NAA, IBA and IAA indicated that IBA at 0.2mg/l level was optimum for rooting. About 70% of the cultures established in *in vivo* conditions.

Gayathri *et al.* (2005) reported encapsulation and regeneration of aseptic shoot buds of turmeric. Praveen (2005) obtained efficient micropropagation and plant regeneration from callus using TDZ at concentrations of 0.1–0.5µM in varieties *Prabha* and *Prathibha*. Direct plant regeneration was observed from leaf tissues without intervening callus under higher concentration of TDZ and the study revealed the occurrence of cytotype and morphological variants among callus regenerated progenies. RAPD analysis also indicated differences within the regenerants.
Prathanturarug et al. (2005) reported a rapid in vitro micropropagation technique using TDZ supplemented liquid medium. They concluded that C. longa bud explants pre-cultured for one week in MS liquid medium supplemented with 72.64µM TDZ then cultured in MS solid growth regulator free medium for 8 weeks, produced maximum shoots (11.4±1.7)/explants, additional cycles of TDZ pre-treatment could be added after 4 weeks of culture on growth regulator free medium.

Micropropagation provides a rapid, reliable system for the production of large numbers of genetically uniform plantlets. It offers a method to increase valuable genotypes rapidly and expedite the release of improved varieties. In addition, micropropagation ensures mass production of elite clones from hybrid or specific parental lines. Micropropogation ensures healthy seedlings with desirable characters (Hiremath, 2006). Tyagi et al. (2007) reported some low cost media for in vitro conservation of turmeric. The low cost media were prepared by replacing the carbon source sucrose with market sugar and gelling agent agar with isubgol (3–5%). Number of shoots produced and survival during conservation was more in media gelled with isubgol.

Renjith and Valsala (2007) optimized the media combination for the development of in vitro pollinated ovules to mature seeds in turmeric. Half strength MS medium supplemented with lower concentrations of auxin or cytokinin induced some ovule development. The combination of ½ MS with 3% sucrose, 0.5mgL⁻¹ NAA and 1mgL⁻¹ BAP showed maximum ovule swelling in cultures. The combination of 4mgL⁻¹ BA along with 0.2mgL⁻¹ IAA produced callusing.
Vijayasree and Valsala (2007) developed an *in vivo* crossing technique between two short duration types *VK 70* and *VK 76* to produce high yielding variety with high curcumin content and curing percentage. Seed set was done by *in vivo* pollination. The seedlings germinated were micropropagated and multiple shoots were obtained in ½ MS medium supplemented with 3% sucrose, 2.5mg{l}^{-1} BA and 0.5mg{l}^{-1} NAA.

Babu *et al.* (2007) reported detailed micropropagation techniques in turmeric using various explants. Panda *et al.* (2007) reported a protocol for *in vitro* propagation of an elite genotype of *C. longa* (cv. *Roma*) using axillary buds from unsprouted rhizomes. Among various media combinations tried by using various concentrations of BA, Kinetin, NAA, IAA and Adenine sulphate, media containing 3mg{l}^{-1} BA was found to be optimum for micropropagation and conservation of *C. longa* plantlets. The genetic uniformity of the somaclones was confirmed by cytophotometry analysis and RAPD techniques. The micropropagated plants need acclimatization under controlled conditions for few days and maintenance in a greenhouse till planted out (Chougule *et al*., 2011). In the field condition, it was observed that the percentage of establishment is less compared to microrhizome induced plants.

Naz *et al.* (2009) reported a rapid propagation and acclimatization method for three varieties of turmeric. They used 70% ethanol in surface sterilization and 5mg{l}^{-1} PPM (plant preservative mixture) in medium for establishment of aseptic culture. Varietal difference in response was reported and among different media combinations, 3mg{l}^{-1} BA alone or 2mg{l}^{-1} BA and 1mg{l}^{-1} NAA in combination
showed more response depending on the variety. During hardening 70–80% survival was obtained in a mixture of sand, soil and peat in the ratio 1:1:1.

Srirat et al. (2009) reported shoot multiplication coupled with rooting in *C. longa* using rhizome buds in MS medium supplemented with 60g/l sucrose and 5mg/l TDZ. Viu et al. (2009) studied the effect of exogenous polyamines (mixture of Putrescine:Spermine:Spermidine, 1:1:1), NAA or BA on turmeric explants in inducing callus formation and subsequent organogenesis. *C. longa* buds in MS medium containing 2mg/l NAA and 5/10mM/l polyamine produced well developed callus with numerous roots. The medium with 10mM/l polyamine and 8.8µM/l BAP produced vigorous plants and excellent shoot formation. The effects of light and cytokinin on micropropagation and microrhizome formation were investigated by Hashemy et al. (2009) and promotive effects of low light intensity for micropropagation and that of short day condition for well developed microrhizome induction was confirmed.


### 2.2.2.14. Microrhizome induction

Nayak (2000) reported an *in vitro* microrhizome induction technique in four cultivars of turmeric viz. *Ranga, Rashmi, Roma* and *Surama* using MS liquid medium supplemented with various concentrations of BA, sucrose and photoperiod.
Concentrations of 3mgl$^{-1}$ BA, 60gI$^{-1}$ sucrose with 4h photoperiod was found to be most effective medium for microrhizome induction. The microrhizomes harvested after 120 days of culture, could be stored in MS media with low concentration of BA (0.01mgl$^{-1}$) and in moist sand at room temperature.

Shirgurkar et al. (2001) reported different factors like sucrose concentration and strength of MS basal medium, affecting microrhizome production in turmeric. Among different concentrations tried, half strength MS basal medium with 8% sucrose was found to be optimum for microrhizome induction. In this study, they also reported the effect of size of multiple shoots on size and weight of microrhizomes, i.e., larger multiple shoots (~15cm) produced larger microrhizomes and under in vivo condition also, the survival depends on the size of microrhizome.

Geetha (2002) reported multiplication and microrhizome induction in turmeric. Multiplication was carried out in the medium supplemented with 1mgl$^{-1}$ BA and 0.5mgl$^{-1}$ NAA (with 90% response). Increased concentration of sucrose (12%) induced microrhizome formation and microrhizomes were successfully established in the field. The applicability of microrhizomes as a suitable material for conservation of germplasm was also described.

An improved microrhizome induction system in *C. longa* was developed by Islam et al. (2004) using rhizome bud explants on MS medium supplemented with different concentrations of BA and Kinetin and NAA at dark and light conditions and different sucrose concentrations. The maximum response was observed in medium containing 12µM BA and 0.3µM NAA and the regenerated plants could be planted out without acclimatization.
Nayak and Naik (2006) reported the effect of certain factors such as concentrations of sucrose and BA in the medium, as well as photoperiod and their interaction in the induction of microrhizomes in turmeric. Medium containing 13.3µM BA and 60g/l sucrose with 4h photoperiod was found to be most effective for microrhizome induction.

Cardenas et al. (2007) reported that during microrhizome induction in Curcuma, the plantlet growth was improved by adding BAP, Kinetin and coconut water to the MS medium. Microrhizomes were induced by increasing the sucrose concentration (6–9%) and under 4h photoperiod. The bud growth was supported by adding ABA to the medium.

Cousins and Adelberg (2008) studied in vitro microrhizome induction in turmeric by culturing in a medium containing BA and jasmonic acid for 6 weeks in small vessels and 23 weeks in larger vessels. In short term course, the biomass accumulation was higher in medium with 1µM BA and without jasmonic acid. In long term course, the concentrations of BA and jasmonic acid did not influence the growth rate.

2.2.2.15. Field evaluation

Salvi et al. (2002) reported field evaluation of micropropagated turmeric plants with 95% successful establishment. The significant increase in shoot length, number of tillers, number and length of leaves, number of fingers and total fresh rhizome weight per plant compared to conventionally propagated plants were observed and RAPD analysis did not show any polymorphism.
Cousins and Adelberg (2008) reported direct relationship of sucrose and dry weight of rhizome produced. They found that 1.8g of sucrose can increase the dry weight up to 1g (i.e., about 59% of sucrose was converted to dry weight), according to them plants grown in a medium with 6% sucrose showed best field establishment.

2.2.2.16. Phytochemical screening

Paramasivam et al. (2008a; 2008b) reported the curcuminoid standardization in turmeric using HPTLC technique. The turmeric powders (1g) were extracted with methanol (20ml×3 times) for 15 minutes and filtered. This was made up to 20ml using methanol. The mobile phase was prepared using chloroform and methanol (48:2) with highest sensitivity at 425nm. The recovery rates were 97.3%, 92.9% and 95.45% respectively for curcumin, demethoxy curcumin and bis-demethoxycurcumin.

Pothitirat and Gritsanapan (2006) evaluated the contents of essential oil and total curcuminoids in dried powder of *C. longa* rhizome collected from north, northeast, central and south Thailand during January to April 2005. The highest content of essential oil (8.20±1.66% w/w) was found in samples from the north and higher curcuminoid (8.99±0.83% v/w) content was reported in samples collected from southern region. This information can be used as guidance for standardization of *C. longa* powder and extracts, and finding the source of good quality of *C. longa* in Thailand.

Saladini et al. (2009) explained a low cost synthetic pathway for obtaining glucosyl-curcuminoids as water soluble potential drugs. The complete 1H and 13C
NMR characterization of naturally occurring curcums, bis-demethoxy curcumin and new synthetic glucosyl curcuminoids was reported.

2.2.2.17. Molecular markers in turmeric

Tyagi et al. (2007) confirmed the genetic stability of 12 months old in vitro conserved turmeric plantlets. Syamkumar and Sasikumar (2007) developed genetic fingerprints of 15 Curcuma species, using Inter Simple Sequence Repeats (ISSR) and Random Amplified Polymorphic DNA (RAPD) markers to elucidate the genetic diversity or relatedness among the species. This study pointed out the limitations of conventional taxonomic tools for resolving the taxonomic confusion prevailing in the genus and suggests the need of molecular markers in conjunction with morpho-taxonomic and cytologic studies while revising the genus.

Panda et al. (2007) reported genetic stability assessment of micropropagated turmeric plants using RAPD analysis and the study revealed monomorphic bands in all the in vitro grown plants.

Molecular analysis based on polymorphism of the nucleotide sequence of chloroplast DNA (cp DNA) was performed inorder to distinguish four Curcuma species viz., C. longa, C. aromatica, C. zedoaria and C. xanthorrhiza. Ninteen regions of cp DNA were amplified successfully via polymerase chain reaction using total DNA of all Curcuma plants. Using intergeneric spacer all four Curcuma plant species were correctly identified (Minami et al., 2009).
2.3. OBJECTIVE OF THE PRESENT STUDY

The major production constraint in ginger and turmeric is the presence of certain soil born and rhizome born diseases which negatively affect the quality and quantity of rhizome. The protection of crop from these pathogens can be done only through some crop improvement technologies. However, the crop improvement program in both these crops is constrained due to no or low seed set. Hence some modern technologies like clonal selection, mutation breeding and induction of polyploidy are used for the improvement of these crops. Propagation by *in vitro* induced microrhizomes, produced independent of seasonal variations is an ideal method both for the production of disease free planting material and for the conservation and exchange of germplasm.

The main objective of the present study was to develop an efficient protocol for large scale microrhizome production in three high yielding varieties of ginger (IISR *Mahima*, IISR *Rejatha* and IISR *Varada*) and turmeric (IISR *Alleppey Supreme*, IISR *Prabha* and *Lakadong* cultivar). The present investigation also envisaged the development of minirhizome technology. The study utilizes anatomical and histochemical tools to confirm the formation of microrhizomes, comparative field evaluation of microrhizome derived plants, minirhizomes and micropropagated plants with that of conventional seed rhizomes. The study further includes RAPD analysis to confirm the genetic fidelity of microrhizome induced plants and also different analytical tools like TLC, HPLC and GC for the comparative qualitative analyses of microrhizome raised plants. Finally the study attempts to confirm the pathogen free nature of the microrhizome induced plants using *in vitro* pathological screening by disc culture method.