Introduction
1. Introduction

1.1. *Garcinia*

The genus *Garcinia* belongs to the family Clusiaceae. It includes more than 250 species (Jones 1980; Stevens 1980; Sweeney and Rogers 2008) and is distributed throughout the tropical Asian, African and Polynesian countries (Ridley 1922; Whitmore 1973; Saleh 2006). In Asia, the distribution of *Garcinia* species is centralized in the Malaysian region and some species are found in India and Micronesian Islands. About 40 species of *Garcinia* produce edible fruits (Yapwattanaphum *et al.* 2002). In India, it is found in the tropical humid evergreen forests of the Western Ghats, Andaman and Nicobar Islands and North Eastern states of India (George 1988). Around 30-35 species of *Garcinia* have been reported in Indian subcontinent by various authors (Anonymous 1956; Maheshwari 1964; Bhat *et al.* 2005). Out of which 7 are endemic to the Western Ghats, 6 to Andaman and Nicobar Islands, and 6 to North Eastern India. The plants in this genus are called saptrees, mangosteens, kokum, garcinias or monkey fruit (Chandran 1996). The most commonly found *Garcinia* species in Western Ghats are *G. gummi-gutta* (Syn. *G. cambogia*), *G. indica*, *G. morella*, *G. xanthochymus*, *G. spicata*, and *G. cowa*.

The *Garcinia* species includes small or medium sized trees, occasionally shrubs and rarely large trees reaching up to 30 m in height. The crown of these trees is monopodial, dense and often conical. The bark is dark brown to black, smooth or sometimes adherent, scaly. Yellow or greenish or occasionally white sticky resinous exudates flow copiously. Leaves are opposite, without stipules, petiolate, entire, leathery to papery and glabrous. Secondary veins are usually prominent, oblique to perpendicular to the midvein. These plants are functionally dioecious. Flowers are solitary, fascicled, racemose or panicked, axillary or terminal, with white, yellow or red colored flowers with 2-6 sepals and petals. The male flowers have numerous stamens which vary in their arrangement and structure and often found with pistilode. The female flowers are sometimes found with staminodes. Stigma is mostly big and conspicuous, free or connate and with as many as numbers of ovules. Ovary 4-12 chambered, with one ovule in each chamber. Fruits are fleshy to woody berry, usually smooth or sulcate with leathery to thin exocarp seated on a persistent calyx. Seeds are
one to several in number, usually large, embedded in an endocarpic pulp (Whitmore 1972; Ridley 1922; Xiwen et al. 2007; Watt 1972).

The species of *Garcinia* are economically important and have found various uses in culinary, cosmetics and pharmaceutical industry. The biological properties of fruits, leaves, stem and barks obtained from different *Garcinia* species have been used in the various traditional practices and have also attracted the attention of biochemists and health practitioners for drug development.

Few species of *Garcinia* are used in ethnobotany and ethnomedicine. Most fruits of *Garcinia* species are used as a food source. *G. mangostana* is one of the best tasting fruits (Morton 1987) and is marketed as a “super fruit” due to its health benefits (Chin et al. 2008). It is the most treasured fruit of the Clusiaceae family (Alexander 1984) and is famed as the queen of tropical fruits (Fairchild 1915, Lim 1984). The pericarp of *G. mangostana* is used in Thai traditional medicine for the treatment of wounds, ulcers and dysentery (Farnsworth and Bunyapraphatsara 1992). In Thai folk and Indian traditional medicine, the fruits of *G. xanthochymus* are used for bilious condition, diarrhea and dysentery (Perry 1980; Ambasta 1986). The fruits are found to be anthelmintic, cardiotonic, alexipharmic, and help to improve appetite. The ‘sherbet’ (fruit juice) made from “Amsul” (sun dried slices of the fruit) is administered in the bilious condition (Yusuf et al. 2009). In traditional Chinese Dai medicine, it is widely used for expelling worms and removing food toxins (Lin et al. 2003). The fruits are extensively used for the preparation of preservatives, jams, curries, beverages, vinegar or as a flavoring in other foods (Martin et al. 1987; Facciola 1998; Baggett et al. 2005; Rai and Anu Appaiah 2014). The sap is used as water color and as a yellow fabric dye (Mabberley 1993). The fruits of *G. spicata* are also used traditionally to dye silk (Konoshima and Ikeshiro 1970). *G. kola* is used by traditional healers for treating illness and as a chewing stick to maintain dental health (Han et al. 2005; Taiwo et al. 1999). Few species are cultivated either for its fruits, vegetables, medicines or for other domestic uses. Young leaves of some *Garcinia* species are eaten cooked by some tribes in the North Eastern regions of India (Arora 1981, Rao and Shanpru 1981). The latex of *G. cowa* is used in Thai folk medicines as an anti-fever agent (Pattaung et al. 1994). Some species are used for curing childhood medication, for menstrual problems, dysentery and fever in traditional medicine.
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(Burkill 1935), and for the potential treatment of HIV (Rukachaisirikul et al. 2003), and cancer (Nabandith et al. 2004).

The genus comprises of plants with potential therapeutic agents. The plant parts have been used for various ethnomedicine practices for the treatment of various disorders like arthritis, cancer, inflammation, malaria, microbial infection, obesity, and oxidative stress. The pharmaceutical industry has isolated, characterised, and evaluated various novel bioactive compounds like hydroxycitric acid (HCA), garcinol, xanthochymol, cambogenol, mangostin, gambogic acid, anthocyanins, and other caged xanthone derivatives for the production of drugs (Pandya et al. 2009; Obolskiy et al. 2009; Acuna et al. 2009).

1.1.1. Garcinia gummi-gutta

Garcinia gummi-gutta (L.) Roxb. (Syn. G. cambogia) commonly known as ‘Malabar tamarind’ is a tree species native to India, Nepal, and Sri Lanka. However, it has been introduced to subtropical regions of South-eastern Asia also. These trees are predominantly found in semi-evergreen to evergreen forests of Western Ghats of India. The medium sized trees grow well both on hilltops and plain lands and reach up to a height of about 25 m. The trees start flowering during summer season (March-May) and fruiting during the rainy season (June-August).

G. gummi-gutta is considered as one of the most important medicinal plants of Clusiaceae family. The different parts of the tree have found various applications in traditional culture in culinary practices, medicines, and pharmaceutical industry. The sun dried rinds are used as a spice, especially in fish curries. It is also used as a substitute for kokum rind. The rind is used to treat rheumatism, bowel complaints, and is employed as a purgative, anthelmintic, emetic, hydragogue, and for the treatment of mouth disease of cattle (Abraham et al. 2006; Lim 2012; Anilkumar et al. 2002; Jena et al. 2002). Tonic prepared from the fruit rind are used for the treatment of heart diseases (Burdock et al. 2005). The rind is also used for rinsing gold, and silver articles. The gum is used as a varnish, and the resin is used for miniature painting (Abraham et al. 2006).

The phytochemistry of G. gummi-gutta has not been extensively studied. However, preliminary phytochemical studies have shown the presence of alkaloids,
flavonoids, phenolic compounds, saponins, tannins, carbohydrates, and proteins (Subhashini et al. 2011). The plant is a rich source of various phytochemicals especially xanthones, benzophenones, organic and amino acids (Inuma et al. 1998; Masullo et al. 2008, 2010; Jena et al. 2002; Kuriyan and Pandya 1931; Mahapatra et al. 2007).

The extracts and pure compounds isolated from *G. gummi-gutta* especially from the fruits showed various biological activities. Studies have shown appetite-suppressant, anti-obesity, hypolipidaemic, anti-diabetic, anti-inflammatory, antioxidative, hepatoprotective, anti-cancer, anti-ulcer, anti-cholinesterase, anti-microbial, anthelmintic, and diuretic activities (Semwal et al. 2015).

1.1.2. *Garcinia indica*

*Garcinia indica* Choisy (Syn. *Brindonia indica*) commonly known as ‘Kokum’ is an indigenous tree of India (Chandran 1996; Padhye et al. 2009). It is found only in the Western Peninsular coastal regions adjoining the Western Ghats of India. The trees are normally found growing along the riverside’s, forests, wastelands. The trees are now being cultivated for its fruits. The medium sized trees grow up to a height of 15-20 m and start to flower during winter (November-February) and bear fruits during summer season (March-May).

*G. indica* has been sourced for various traditional cultures, culinary uses, medicine, cosmetics, and pharmaceutical industry. It is used as a culinary agent and is used as an acidulant for curries. The syrup obtained from the rind is used as a cooling drink (Shenoy 1989; Menezes 2000, 2002; Padhye et al. 2009). It is also used for the preparation of chutneys, pickles (Menezes 2000, 2002), and also for the preparation of soup known as Kokum kadi. The kadi is supposed to be digestive and relieve gastric problems (Shenoy 1989; Menezes 2000, 2002). Industrially, the fruits are used for the preparation of concentrated syrup, which is used as a cool drink. Kokum butter isolated from the seeds is used in confectionery, medicines, and cosmetic industry (Reddy and Prabhakar 1994). The butter is also used in the production of soaps, and candles (Bhat et al. 2005; Nayak et al. 2010).

Proximate and phytochemical analysis has shown that the fruit rind of kokum contains proteins, tannin, pectin, sugars, and fats (Krishnamurthy et al. 1982; Nayak
et al. 2010). The seeds are rich in stearic, oleic, and stearic triglycerides (Dushyantha et al. 2010). The plant is also a rich source of various phytochemicals (Padhye et al. 2009).

The extracts and isolated compounds showed various pharmacological activities. Studies have reported antibacterial, antifungal, antioxidant, anti-lipid peroxidation, neuroprotective, gastro protective, anti-aging, anti-obesity, cardio protective, anti-diabetic, anti-neoplastic, and chemopreventive activities (Baliga et al. 2011).

1.1.3. *Garcinia morella*

*Garcinia morella* commonly known as ‘Mysore gamboge’ is found in India, Sri Lanka and Philippines. The trees are predominantly found in the evergreen to semi-evergreen forests. The trees grow up to a height of 12 m and flower during winter (October-December) and bear fruits during summer (March-May).

The fruits are used as a culinary agent. The ripe fruits are eaten and often preserved by drying the slices and by making pickles. Traditionally the dried fruits are used as a remedy for dysentery, gastritis, and diarrhea and as an anti-inflammatory drug in ayurveda. The plant is a rich source of various phytochemicals like morellin, isomorellin, moreollin, and isomoreollin (Narasimha Rao et al. 1954; Sani 1967).

1.2. Sexual systems in *Garcinia* species

The species of *Garcinia* displays a great variation in its floral morphology (Whitmore 1998; Lee et al. 2002) that has led the researchers to find a large variation even in its sexual system. The previous reports provided by earlier researchers confirm that the *Garcinia* is a dioecious species (Corner 1952; Jansen 1991). Some authors concur that this genus to be dioecious (Sweeney 2008), but some others consider that it is a gynodioecious (Pangsuban et al. 2007), androdioecious (Berg 1979) and monoecious (Cavalcante 1996). However, Thomas (1997) reports that the *Garcinia* species of South Asian forests has female-biased sex ratio compared to the majority of the dioecious tropical trees which exhibit male-biased sex ratio (Thomas 1996; Opler and Bawa 1978). The existence of female-biased sex ratio in their reproductive behavior is due to the phenomenon of agamospermy (Thomas 1997; Richards 1997a; Ramage et al. 2004).
The occurrence of apomixis in species of *Garcinia* is common and is reported by many authors (Gustafsson 1946; Horn 1940a, b; Maguire 1976; Richards 1990a, b, c, 1997a, b; Sprecher 1919; Thomas 1997). Many species like *G. mangostana* (Kaur et al. 1978), *G. parviflora* (Ha et al. 1988), *G. scortechinii* (Thomas 1997), *G. livingstonei* (Puri 1939), and *G. hombroniana* (Richards 1990b) are reported to be apomictic. However, *G. atroviridis* shows facultative apomixis (Pangsuban et al. 2009). Richards (1997b) has reported that all species of *Garcinia* are not apomictic like *G. cantleyana*. Cases of subdioecy have also been reported in many species, like gynodioecy in *G. indica* (Rawat and Bhatnagar 2005) and androdioecy in *G. gummi-gutta* (George et al. 1992). Sharma et al. (2012) have reported *G. xanthochymus* (especially in North Eastern region of India) to be monoecious. In some species like *G. forbesii* (Ramage et al. 2004) and *G. brasiliensis* (Leal et al. 2013) both monoecy and dioecy have been reported.

Among a large variety of angiospermic plants, the species, *Garcinia* exhibits a huge variation in its floral structure. Most of the *Garcinia* species have four sepals and petals but in some cases, two, three, five or many perianth lobes occur per whorl and in some, the perianth lobes are completely fused to each other in the bud. The staminodes in the pistillate flowers can be fasciculate or non-fasciculate and phalangiate or free, in the degree of fusion with each other when clustered or in the fusion with the petals. The anthers also vary among species in their shape of thecae, number of locelli per anther and whether these locelli are present or absent. In these pistillate flowers, style and style branches may be present or absent. However, the ornamentation of stigma is highly diverse. Some species have additional structures called fasciclodes and it has been interpreted that they are sterile reproductive structures or as of receptacular origin (Pierre 1883; Leins and Erbar 1991) and may be a disk, lobe or ring shaped (Robson 1972; Jones 1980; Stevens 2006).

The extreme diversity in the floral form of *Garcinia* species has led to a reliance of floral characters, thereby delimiting this genus by earlier workers and leading to the framing of infrageneric classifications. Various authors have recognized several segregate genera based largely on the floral morphology (Planchon and Triana 1860; Bentham 1862; Engler 1893, 1925; Vesque 1893; Perrier de la Bâthie 1948, 1951). These genera include *Ochrocarpos* Thouars, *Pentaphalangium* Warb., *Rheedia* L. and *Tripetalum* Schumann. However, despite the differences existing in these
genera, various authors have suggested that these genera should be included under *Garcinia* (Stevens 2005, 2006; Jones 1980; Robson 1958; Turner and Stevens 1999).

### 1.3. Sex determination

Sex determination or expression has been a thriving area of research for botanists. Sex is an important factor within a species that leads to differences in behavior, physiology, and economic importance of a species owing to their cultivation and plantation in large numbers. Understanding and recognition of female and male plants not only helps to develop strategies for increasing the commercial harvest, but also helps in understanding the biology of unisexual species. Studies related to dioecy have enabled researchers to assess the factors determining the sexual phenotype, mechanism of sex determination, evolutionary, and developmental pathways (Aryal and Ming 2013; Dellaporta and Calderon-Urrea 1993; Lebel-Hardenack and Grant 1997; Marziani *et al.* 1999; Matsunaga 2006; Matsunaga and Kawano 2001; Vyskot and Hobza 2004). Sex in any reproducing species helps in ensuring its continuation of generation and genetic improvement through breeding.

In higher plants, the mechanism of sexual dimorphism differentiates the male and female individuals from each other. This mechanism leads to the distinction of forms with respect to their sex organs. In approximately 10% of higher plants, such amplitude of unisexuality persists and can be separated into two major classes i.e. monoecy and dioecy. The sexually dimorphic phenomenon, i.e. dioecy is thought to be a recently evolved phenomenon from hermaphroditism (Ross 1980) and is characterized by the presence of separate male and female flowers on two separate individuals. From the evolutionary point of view, dioecy has been originated prevalently through gynodioecious and the monoecious pathway (Webb 1999).

### 1.4. Sex-linked markers in dioecious plants

Identification of sex in commercially important dioecious crops is very important because the sex of the plant plays a significant role in determining the economic value and also helps to modulate cultivation practices (Tanurdzic and Banks 2004). In dioecious crops like *Simmondsia chinensis* (Agrawal *et al.* 2007), the female plants produce yields of commercial importance. However, in others like *Cannabis sativa* (Sonoda *et al.* 2003) male plants are preferred over the female plants. In contrast to
this, in some plants like *Carica papaya* (Urasaki *et al.* 2002) hermaphrodites are favored over male and female plants. Since in most dioecious crops reproductive stage starts after 1 to 15 years, identification of the sex at juvenile stage is difficult. If this problem faced by the breeders especially while selecting the superior parental types is tackled by eliminating commercially unwanted sex that would help the breeders to reduce the field space, time, and other resources in maintaining commercially unwanted sex. Realizing this problem many researchers have tried to develop various strategies for the selection of desirable gender. In order to differentiate sexes, various marker systems like morphological, cytological, physiological and molecular markers were developed.

### 1.4.1 Morphological markers

Morphological characters like the size of leaf, nature of stems, branches, inflorescences, and flowers were examined to differentiate between the sexes (Dzhaparidze 1969; Geber *et al.* 1999; Li *et al.* 2006). However, these characters may alter the environment and climatic conditions. Hence, these parameters are not reliable. Moreover, identification of the sexes in the vegetative stage is impossible and can only be made when the plant reaches reproductive stages.

### 1.4.2. Physiological markers

Physiological parameters between male and female plants differ from each other as both male and female plants are destined to perform different functions. Researchers have investigated the physiological differences in the gross developmental changes, pigmentation, photosynthetic activity, phenolic content, and respiration rate between male and female plants (Dzhaparidze 1969; Geber *et al.* 1999; Li *et al.* 2006). However, these parameters tend to change during the growth and the developmental stages of a plant. The availability of limited differences and the influence of environmental factors on the physiological processes make it unreliable for identification of sex at seedling or juvenile stages.

### 1.4.3. Cytological markers

With the advent in the discovery of sex chromosomes in dioecious species, several investigations have been carried out to determine the chromosome mechanism as the sex- determining genes present on the chromosomes are responsible for the
differences in morphological characters of different sexes. Researchers have identified various sex chromosome complexes like (a) XX female and XY male in *Melandrium album* (Blackburn 1923) and *Humulus lupulus* (Jacobson 1957); (b) XX female and XY<sub>1</sub>Y<sub>2</sub> male in *Humulus japonicus* (Winge 1923; Jacobson 1957), *Rumex acetosa* (Kihara and Ono 1923a, b) and *Rumex hastatulus* (Smith 1955); (c) XY female and XX male in *Fragaria elatior* (Kihara 1926) and (d) XX female and XO male as in *Dioscorea sinuata* (Meurman 1925). However, in most of the plants, identification of sex is cumbersome since the chromosomes do not differ morphologically from autosomes or from each other like in *Asparagus officinalis* (Michalik et al. 2009) or in some cases the X and Y chromosomes are too small to distinguish as in case of *Actinidia deliciosa* (Shirkot et al. 2002). In some, the ratio of X chromosome and autosomes determines the sexes eg. *Rumex acetosa* (Ainsworth 2000). Identification of sex in such species is, therefore, difficult and hence requires other parameters like sequence data for the determination of sex.

### 1.4.4. Biochemical markers

Isozyme polymorphism is widespread in many plants and is useful in developing biochemical markers for the identification of sex in dioecious crops (Dzhaparidze 1969; Sharma et al. 2010). These enzymes differ only in the amino acid sequence but catalyze the same chemical reaction (Markert and Moller 1959). Differences in the isozymes patterns and protein profiles is obtained by using gel electrophoresis techniques and resolved using enzyme specific strains for the identification of the sexes in dioecious species like *Actinidia chinensis* (Khukhunaishvili and Dzhokhadze 2006), *Asparagus officinalis* (Maestri et al. 1991), *Cannabis sativa* (Truta et al. 2002), *Hippophae rhamnoides* (Sharma et al. 2010), *Mercurialis annua* (Kahlem 1976) and *Phoenix dactylifera* (Qacif et al. 2007; Bekheet et al. 2008). Since isozymes are affected by environmental conditions and their expression varies from tissue to tissue, phenological stages of plant, post-transcriptional modification, and the requirement of different protocol for each enzyme system limits its use in identification of sex.

### 1.4.5. Molecular markers

Most dioecious crops reach their reproductive maturity after 1-15 years. External morphology of their embryonic and juvenile stages does not suffice for the
determination of sex. In the absence of distinct sex chromosomes and inadequate biochemical markers, it is highly difficult to distinguish the sexes of the plants. Molecular marker techniques based on DNA and RNA serve as potential tools for the differentiating the sexes by the breeders and thus helping the breeders in saving space, time, and other resources in maintaining the undesired plants till flowering (Agrawal et al. 2007) and gain commercial benefits.

Isolation of good quality genomic DNA from male and female plants is crucial for sex determination using molecular markers. Molecular marker techniques like RFLP, RAPD, AFLP and ISSR have been used for this purpose. However, locus-specific markers like SCARs are designed by cloning and sequencing of the desired fragments.

Restriction Fragment Length Polymorphism (RFLP) was the first molecular marker system developed in the early 1980s (Botstein et al. 1980). In this technique, restriction enzymes like Eco R1 and Hind III digest the genomic DNA at specific sequences; and the digested DNA is electrophoresed, blotted using a membrane and tagged using a radio labeled probe. RFLP technique helps to detect the differences between two individuals by comparing the DNA sequences of the same loci. Changes within these sequences can result in DNA fragments differing in length and molecular weights. These changes may be due to point mutations, insertions, inversions, translocation, transposition, or deletions (Weising et al. 2005; Edwards and Mc Couch 2007). RFLP technique was employed to identify the molecular probe d47 for sex typing in Asparagus officinalis. The DNA samples were single digested with various restriction enzymes. The distance between the marker and the sex determinants was estimated to be 6.9 cM (Biffi et al. 1995).

Randomly Amplified Polymorphic DNA (RAPD) markers are PCR-based markers which amplify random DNA segments with single, typically short primers of an arbitrary nucleotide sequence (Williams et al. 1990). During PCR reaction a single species of primer anneals to the genomic DNA at two different sites on complementary strands of template DNA. The amplification products can be separated by gel electrophoresis and visualized using UV gel documentation system. The RAPD technique is fast, efficient, and reliable. It uses a small amount of DNA, no prior knowledge of sequence is not required and does not involve radioactive
assays (Kiss et al. 1993), species-specific probe libraries and blotting. However, these dominant markers suffer from problems of repeatability (Liu et al. 1994) and a mismatch of a single nucleotide can prevent the primer from annealing and the loss of band.

RAPD technique has been largely applied for the determination of sex in dioecious crops. Sex determination using RAPD marker was first reported by Mulcahy et al. (1992) in *Silene latifolia*. Later, many workers have identified markers which were able to differentiate between male and female plants. Sex was successfully identified in *Pistacia vera* (Hormaza et al. 1994), *Humulus lupulus* (Polley et al. 1997), *C. simplicifolius* (Yang et al. 2005), *Cannabis sativa* (Sakamoto et al. 1995; Mandolino et al. 1999), *Actinidia delicosa* (Shirkot et al. 2002), *Phoenix dactylifera* (Younis et al. 2008), *Simmondsia chinensis* (Agrawal et al. 2007), *Piper beetle* (Samantaray et al. 2012), *Mercurialis annua* (Kafkas et al. 2001), *Momordica dioica* (Baratakke and Patil 2009), *Myristica fragrans* (Shibu et al. 2000), and *Trichosanthes dioica* (Singh et al. 2002; Kumar and Sinha 2012).

Amplified Fragment Length Polymorphism (AFLP) marker technology was developed to overcome the limitations of reproducibility associated with RAPD (Vos et al. 1995). AFLP combine the features of RFLP along with the flexibility of PCR by ligating primer recognition sequences to the restricted fragments using a limited set of primers. The technique of AFLP involves restriction digestion of genomic DNA with two restriction digestion enzymes, a rare cutter (*EcoR1, Pst1, Hind III*) and a frequent cutter (*Mse1, Taq1*). The adaptors are then ligated to both ends of the fragments to provide known sequences for PCR amplification. The double-stranded oligonucleotide adaptors are designed in such a way that the restriction site is not restored after ligation. Therefore, only the fragments which have been cut by the fragment cutter and rare cutter will be amplified. The fingerprint contains 50-100 AFLP fragments which can be separated by high-resolution electrophoresis systems. These fragments in the gel based or capillary DNA sequence can be detected by fluorescent or by radio labeling the primers. In brief, the genomic DNA is digested using *Eco RI* and *Mse I* for about 5-6 h at 37 °C, and then it is heated to 60-70 °C to inactivate the enzymes. Ligation of specific adaptors to restriction fragments is performed by using adaptor mixture and incubation for 20 h at 20 °C, and the ligation mixture product is diluted for 10 times. PCR pre-amplification is carried out using
pre-amplification primer mixture, PCR reaction buffer, Taq DNA polymerase, and diluted ligation mixture. After PCR, the pre-amplified products are diluted 45 times, which is followed by amplification using a selective primer combination for selective amplification. The PCR is carried out by using PCR buffer, selective primers, Taq DNA polymerase, and diluted pre-amplified PCR products. The PCR products after amplification are separated on 6% denaturing polyacrylamide gel. Since AFLP technique has a high multiplex ratio, it is relatively reproducible and does not require sequence information prior to fingerprinting but in these markers, polymorphic information content in the bi-allelic marker is low and requires a high-quality DNA for complete restriction digestion. Reamon-Buttner et al. (1998) first suggested that the technique of AFLP could be used for map-based cloning of sex determining genes in Asparagus. Later, other workers also generated sex-linked markers in Asparagus using AFLP markers (Reamon-Buttner and Jung 2000; Jamsari et al. 2004; Nakayama et al. 2006). AFLP marker for sex determination was developed in Eucommia ulmoides (Wang et al. 2011), Ficus fulva (Parrish et al. 2004), Rumex acetose and Rumex rothschildianus (Rahman and Ainsworth 2004), Rumex nivalis (Stehlik and Blattner 2004), Salix viminalis (Semerikov et al. 2003), S. chinensis (Agarwal et al. 2011), and Uapaca kirkiana (Mwase et al. 2007).

Inter Simple Sequence Repeat (ISSR) markers are semi-arbitrary primers amplified by PCR in the presence of one primer complementary to the target microsatellite (Zietkiewicz et al. 1994). ISSR primers are derived from an arbitrary nucleotide sequence of di and trinucleotide repeats which are based on the ubiquitous presence of SSRs that are distributed throughout genome. The ISSR primers are useful for the detection of variation within the species (Wolfe and Liston 1998) where the sequence information is limited about the organism. The amplification of the primer leads to multilocus and highly polymorphic patterns. ISSR markers have been used for the identification of sex in Calamus tenuis (Sarmah and Sarma 2011), Carica papya (Parasnis et al. 1999; Gangopadhyay et al. 2007; Da Costa et al. 2001), Humulus japonicas (Aleksandrov et al. 2011), Humulus lupulus (Danilova and Karlov 2006), Phoenix dactylifera (Younis et al. 2008), Simmondsia chinensis (Sharma et al. 2008), Heikrujam et al. 2014a, b), and Trichosanthes dioica (Nanda et al. 2013).

Sequence Related Amplified Polymorphism (SRAP) markers are co-dominant and multilocus marker developed by Li and Quiros (2001). The aim of the SRAP
technique is to amplify the Open Reading Frames (ORFs). It is based on two primer amplification using the AT or GC rich cores to amplify intragenic fragments for polymorphism detection (Agarwal et al. 2008). SRAP markers were used for identification of sex in Buchloe dactyloides (Zhou et al. 2011).

In order to overcome the limitations of RAPD, ISSR, and AFLP primers, highly reliable, reproducible, locus specific, and cost effective SCAR primers were developed. Sequence Characterized Amplified Region (SCAR) markers are PCR-based primers that represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification using sequence-specific oligonucleotide primers (Paran and Michelmore 1993). The inception of SCARs involves cloning the amplified products of arbitrary marker techniques and then sequencing the two ends of the cloned products. The sequences, therefore, are used to design specific primer pairs of 15-30 bp which will amplify a single major band of the size similar to that of the cloned fragment. Greater the variation in DNA sequence, the easier it is to generate polymorphic markers. These primers are reliable, reproducible, and less sensitive to reaction conditions, not affected by physical forms or age of the sample and are also not affected by the introns which affect the priming sites. SCAR markers were developed from RAPD, ISSR, and AFLP amplicons to determine the specific trait. SCAR primers were developed for Carica papaya (Parasnis et al. 2000; Niroshini et al. 2008), Eucommia ulmoides (Xu et al. 2004), Phoenix dactylifera (Dhawan et al. 2013), Piper longum (Manoj et al. 2005), Pseudocalliergon trifarium (Korpelainen et al. 2008), Ficus fulva (Parrish et al. 2004), and many other dioecious species for the identification of sex.

Cleaved Amplified Polymorphic Sequence (CAPS) polymorphisms are differences in restriction fragment lengths caused by single nucleotide polymorphisms that create or abolish restriction endonuclease recognition sites in PCR amplicons produced by locus-specific primers. These are co-dominant markers. This technique amplifies the DNA fragments using specific 20-25 bp primers followed by digestion with restriction enzyme and separation of the digested fragments on gel electrophoresis. CAPS markers were used to identify male and female sexes in Simmondsia chinensis (Ince et al. 2010; Ince and Karaca 2011).
RNA fingerprinting based markers like differential display and cDNA-AFLP has also been utilized for the sex determination in *Piper longum*, *Cannabis sativa* and *Trichosanthes dioica* (Manoj et al. 2008; Moliterni et al. 2004; Roy et al. 2008).

Due to the presence of limited morphological, physiological, biochemical, and even cytological characters to determine the sex of dioecious crops at reproductive or at any developmental stage, advanced multidisciplinary DNA/RNA techniques can be used for the identification of sexes in the plants. Since molecular techniques especially SCAR markers are highly reliable, accurate, and reproducible and have proved to be useful for marker assisted selection of male and female plants in dioecious plants, they can be used for the identification and development of sex-specific SCAR markers in *Garcinia* species of Western Ghats.

Hence, the present study was undertaken with the following major objectives to develop a molecular technique to determine the sex of *G. gummi-gutta*, *G. indica* and *G. morella* growing in the Western Ghats region of India.

1.5. Objectives of present research work

1. Survey, identification, selection, documentation of male and female trees of *Garcinia* growing in different regions of the Western Ghats.