Review of Literature
2. Review of literature

2.1. The genus *Garcinia*

*Garcinia* L. belongs to family Clusiaceae (Guttiferae) which is distributed throughout the tropical regions of the world, ranging between the latitudes of 10° North and 10° South (Cox 1976). It is the biggest genus in the family and has approximately 250 species (Jones 1980; Stevens 1980; Sweeney and Rogers 2008). The members of the genus *Garcinia* are evergreen trees or shrubs with yellow, white, and greenish gum resins. Leaves are sub coriaceous or leathery with no stipules. Flowers are solitary, fasiculate, umbelled or panicked with 4-5 sepals which are leathery and persistent. Petals 4 to 5, imbricate. The stamens staminate flowers vary in number. They may be fasiculate or phalangiate or non fasiculate and free in the degree of fusion to each other when clustered and in the degree of fusion to the petals. In pistillate flowers, style branches can be present and absent and the surface ornamentation is very diverse. Some groups have fasciculodes (Robson 1972, Stevens 2006) and have been variously interpreted as sterile reproductive organs (Robson 1972, Jones 1980) or as of receptacular origin (Pierre 1883, Leins and Erbar 1991). Fruit is a berry with fleshy rind enclosing 2-8 large pulpy seeds. The trees are usually dioecious or polygamous.

2.1.1. Origin and Distribution

The genus is thought to have arisen in South East Asia, ranging from the southern part of Thailand and peninsular Malaysia to Indonesia and some parts of Philippines (Richards 1990a, Whitmore 1998), but the exact origin of the genus is still debated. However, the species of *Garcinia* are centralized in the Malaysian region and some species are found in India and Micronesian Islands.

The genus *Garcinia* is distributed across the West Africa to the tropical South East Asia, ranging from southern parts of the Thailand and peninsular Malaysia and Indonesia to the Fiji Islands (Ridley 1922, Whitmore 1973, Sharma *et al.* 1993, Mabberley 2005, Fairchild 1930, Corner 1988). Of all the species of *Gracinia* found, only 30 species are cultivated (Hammer 2001).

2.1.2. Distribution of *Garcinia* species in India

In India, *Garcinia* species are found in the tropical humid evergreen forests of the
Western Ghats, Andaman and Nicobar Islands, and North Eastern states of India (George 1988). 30 species of *Garcinia* have been reported by Anderson in the Flora of British India (Hooker 1874), whereas other authors have reported 35 species (Maheshwari 1964; Bhat *et al.* 2005). 15 species are found in the North Eastern Himalayan region and 11 species are found in the Southern Western Ghats region. 7 out of 11 are endemic to the Western Ghats, 6 out of 15 are endemic to North Eastern India, and 6 are endemic Andaman, and Nicobar Islands. *G. gummi-gutta*, *G. indica*, *G. morella*, *G. atrovirides*, *G. cowa*, *G. lanceaefolia*, *G. hombroniana*, *G. prainiana*, and *G. mangostana* are the most common species found in India.

### 2.1.3. *Garcinia gummi-gutta*

#### Distribution and habitat

*G. gummi-gutta* (L.) Roxb. (Syn. *G. cambogia*) commonly known as ‘Malabar tamarind’ is native to South Eastern Asia. It is restricted to India, Nepal and Sri Lanka, and predominantly found in the evergreen to semi-evergreen forests (Ramesh and Pascal 1997). It grows on hill tops as well as on the plains and preferably near water-logged areas.

#### Traditional uses

The fruits of *G. gummi-gutta* are very important and are used as a spice, especially in fish curries (Samarajeewa and Shanmugapirabu 1983). 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).

#### Phytochemistry

The plant is a rich source of various phytochemicals especially xanthones benzophenones, organic and amino acids. Xanthones isolated from the plant include Garbogiol, Rheediaxanthone, Oxy-guttiferone-I, Oxy-guttiferone-K, Oxy-guttiferone-

Biological activities

The extracts and pure compounds isolated from G. gummi-gutta especially from the fruits showed various biological activities. Studies have shown appetite-suppressant (Rao et al. 2010), anti-obesity (Ranjith et al. 2011), hypolipidaemic (Koshy et al. 2001), anti-diabetic (Hayamizu et al. 2003), anti-inflammatory (Reis et al. 2009), anti-oxidative (Subhashini et al. 2011), hepatoprotective (Mahendran and Devi 2001), anti-cancer (Saadat and Gupta 2012), anti-ulcer (Mahendran et al. 2002), anti-cholinesterase (Subhashini et al. 2011), antimicrobial (Shivakumar et al. 2013), anthelmintic (Mathew et al. 2011a), and diuretic (Mathew et al. 2011b) activities.

2.1.4. Garcinia indica

Distribution and habitat

G. indica Choisy is an indigenous tree of India commonly known as ‘Kokum’. It grows along the Western Ghats, Western peninsular coastal regions as well as parts of Eastern parts of India. The tree is normally found growing along the riversides, forests, wastelands, and in cultivated areas.

Traditional uses

Ethnobotanically 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 and pickles (Menezes 2000, 2002). It is also used for the preparation of soup known as kokum kadi. The kadi is supposed to be digestive and relive 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 kokum butter is also used in the production of soaps and candles (Bhat et al. 2005; Nayak et al. 2010).

**Phytochemistry**

The plant is a rich source of various phytochemicals like Garcinol, Xanthochymol, Hydroxycitric acid, Hydroxyl citrate lactone, Anthocyanin, Cyanidin-3-glucoside, and Cyanidine-3-sambubioside (Padhye et al. 2009).

**Biological activities**

The extracts and isolated compounds showed various pharmacological activities. Studies have shown the antibacterial (Negi and Jayaprakasha 2006; Sang et al. 2001), antifungal (Varalakshmi et al. 2010; Selvi et al. 2003), antioxidant (Yamaguchi et al. 2000), anti-lipid peroxidation (Mishra et al. 2006), neuroprotective (Liao et al. 2005; Lenta et al. 2007), gastroprotective (Chatterjee et al. 2003, 2005), anti-aging (Sahasrabudhe and Deodhar 2010; Bhat et al. 2005), anti-obesity (Jena et al. 2002, Tsuda et al. 2003), cardioprotective (Xu et al. 2004b), anti-diabetic (Kirana and Srinivasan 2010; Sasaki et al. 2007), anti-neoplastic (Balasubramanyam et al. 2004; Ahmad et al. 2010) and chemo-preventive (Yoshida et al. 2005; Tanaka et al. 2000) activities.

**2.1.5. *Garcinia morella***

**Distribution and habitat**

*G. 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.

**Traditional uses**

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 resin is used in food and medicines.

**Phytochemistry**

The plant is a rich source of various phytochemicals like Morellin, Isomorellin, Moreollin, Isomoreollin, Deoxy-morellin, Dihydro-isomorellin, Ethoxydihydro-isomorellin, α1-Guttiferin, α2-Guttiferin, γ- Guttiferinic acid, δ- Guttiferinic acid, β-Guttiferin, Morellic acid, and Isomorellic acid (Narasimha Rao *et al.* 1954; Sanjiva Rao 1937, Sani 1967).

**Biological activities**

Biological activities of the *G. morella* are not extensively explored. However, some studies on the extracts and isolated compounds revealed antioxidant (Boruah *et al.* 2012), anti-cancer (Choudhury *et al.* 2016), and anti-fungal (Sarma *et al.* 2016) activities.

**2.2. Sexual systems in plants**

In angiosperms, the majority of the flowering plants produce perfect flowers with both functional male and female organs in the same flower. However, 10% of the angiosperm species produce unisexual flowers (Yampolsky and Yampolsky 1922). The plant species with unisexual flowers can be divided into two groups, i.e. monoecious and dioecious. In monoecious plants, both male and female flowers are borne on a single individual plant, whereas in dioecious plants, both male and female flowers are borne on separate male and female individual plants, but occasionally in some dioecious species, bisexual plants occur besides male and female individuals. Such a condition is called sub-dioecy, as seen in *Asparagus officinalis* (Machon *et al.* 1995).

Only about 6% of the angiosperm species are reported to be dioecious (Renner and Ricklefs 1995). Many families have high concentrations of dioecious genera like Menispermaceae (100%), Myristicaceae (78%), Moraceae (62%), Urticaceae (52%), Anacardiaceae (50%), Monomiataceae (47%), Euphorbiaceae (39%), and Cucurbitaceae (32%) (Renner and Ricklefs 1995). Likewise, many floras are also rich in dioecious species, such as Hawaii (27.8%) or in New Zealand (12-13%) (Bawa 1980).
Breeding systems in plants exhibit a wide variety ranging from hermaphroditism through various forms of monoecy, to dioecy (Sakai and Weller 1999). A great majority of flowering plant exhibits a common form of breeding system in which 72% of the species are hermaphrodites, 4% are truly dioecious, 7% are either gynodioecious or androdioecious and 7.5% of the plant families have some dioecious members (Yampolsky and Yampolsky 1922).

2.2.1. Sex determination mechanisms in dioecious plants

Sex determination is the developmental decision in the life cycle of the plant that leads to the differentiation of two organs or cells that produce two gametes (Ainsworth 2000). The sexual phenotype in plants can be determined by genetic and environmental factors (Grant 1999). The genetic basis of sex determination in dioecious plants is diverse. Therefore, an extensive theoretical, cytological, molecular, and in silico studies have been carried out over the years for determining the sex forms (Jamsari et al. 2004; Ming et al. 2007, 2011; Moore et al. 2003; Obara et al. 2002; Ma et al. 2004). Wide variety of sex determination mechanisms and its genetics involved in the plant kingdom has been postulated (Aryal and Ming 2013; Charlesworth 2002; Matsunaga 2006; Tanurdzic and Banks 2004; Vyskot and Hobza 2004). The control of expression of sex in dioecious plants is based on three basic sex determination mechanisms viz. genetic, epigenetic, and hormonal.

2.2.1.1. Genetic control

Chromosomes and their sex-determining genes are mainly responsible for the genetic control of sex determination in dioecious plants. According to Aryal and Ming (2013), a vast variation in the genetic control of sex determination is attributed to the DNA polymorphism in the loci that is directly involved in the expression of the particular phenotype. A single or multiple loci, either linked tightly or unlinked on autosomes may be responsible for the genetic sex determination (Grant et al. 1994). The simplest sex determination mechanism involving a single locus is seen in Atriplex garrettii (Ruas et al. 1998) and a relatively complex sex determination where sex determination is caused by multiple unlinked loci is seen in Mercurialis annua (Louis 1989).
The presence of sex chromosomes has been documented in several plant species (Westergaard 1958). There are two types of sex chromosomes (Matsunaga and Kawano 2001) - Homomorphic sex chromosomes, in which the sex chromosomes are indistinguishable morphologically from each other (Mariotti et al. 2008) and heteromorphic sex chromosomes are the chromosomes, which can be distinguished by cytological analysis (Parker 1990). Allen (1917) reported the first plant sex chromosome in liverwort Spaerocarpus donnellii, and later Santos (1923) reported the first sex chromosome in dioecious plant Elodea gigantean. Subsequently, heteromorphic sex chromosomes have been reported from 20 species belonging to 4 different families and homomorphic sex chromosomes from 20 species belonging to 13 different families (Aryal and Ming 2013; Ming et al. 2011). In general, sex chromosome determination mechanisms are of three types – Active Y chromosome, X chromosome to autosomes ratio and ZZ/ZW ratio.

**Active Y chromosome (XX/XY)**

Active Y chromosome based sex inheritance in dioecious plants has been reported in Silene latifolia and Carica papaya (Westergaard 1958). Females are homogametic with two identical X chromosomes and males heterogametic with one X and a dominant Y chromosome. Sexual dimorphism displayed in Silene latifolia is well studied (Aryal and Ming 2013; Atanassov et al. 2001; Delichere et al. 1999; Filato 2005; Liu et al. 2004; Ming et al. 2007; Moore et al. 2003; Nicolas et al. 2005; Sondur et al. 1996; Westergaard 1948). In contrast to the mammalian sex chromosomes, the X and Y chromosomes are the largest and the second largest respectively in plant genome of Silene latifolia (Ciupercescu et al. 1990; Matsunaga and Kawano 2001). The active Y chromosome contains regions for carpel suppression genes, stamen promoting genes, and maleness determining genes (Charlesworth 2002; Matsunaga and Kawano 2001; Negrutiu et al. 2001; Vyskot and Hobza 2004). Two different types of Y chromosomes, Y and $Y^h$ have been found to be responsible for both maleness and hermaphroditism in the case of Carica papaya. The sex determining locus has been mapped to 14 cM in the papaya genome (Aryal and Ming 2013; Liu et al. 2004; Ming et al. 2007). Since during in vitro technique only monoploid females have been obtained, the X chromosome plays an important role in both male and females (Ye et al. 1991).
X chromosome to autosome ratio (X:A)

In some plants like Rumex acetosa, Humulus japonicans, Humulus lupulus, and Phoenix dactylifera (Ainsworth 2000; Shephard et al. 1999, 2000; Siljak-Yakovlev et al. 1996) sex determination are independent of the presence or absence of morphologically distinct Y chromosome and is controlled by the number of X chromosomes: Autosomes ratio. In Rumex acetosa, aneuploid sex chromosomes are present where the female is homogametic (2A + 2X) and males are heterogametic (2A + X +2Y) (Kihara and Ono 1923a, b). In this species X: A ratio determine the sex, where X: A ratio of 1 results in females, the ratio of 0.5 results in males and intermediate ratio results in intermediate sex (Ainsworth et al. 1999). Analysis of polyploids showed that the X chromosomes contained female determining genes and autosomes with male determining genes (Matsunaga and Kawano 2001).

ZZ and ZW system

This type of sex determination is seen in the case of Fragaria elatior, which resembles the sex determination in birds, where female heterogamety (ZZ male and ZW female) is observed (Correns 1928). A similar type of sex determination is also seen in Myristica fragrans (Flach 1966), Populus spp. (Yin et al. 2008), and Salix viminalis (Sansone 1938).

2.2.1.2. Epigenetic control

Epigenetic or liable sex inheritance is regulated by switching the genes at the transcriptional or post-transcriptional level under the control of various environmental and physiological determinants, altered euchromatin structure, DNA methylation, and insertion of transposons in the genome (Aryal and Ming 2013; Milewicz and Sawicki 2012; Slotkin and Martienssen 2007; Vyskot et al. 1993; Weil and Martienssen 2008). Such type of sex determination is seen in few dioecious species like Carica papaya, Ilex integra, Silene latifolia etc (Aryal and Ming 2013; Korpelainen 1998; Takagi and Togashi 2012; Vyskot et al. 1993). The action of heavy metals like Pb++, Cu++, Zn++, and Ag+ is correlated with the shift in expression of sex (Law et al. 2002; Soldatova and Khryanin 2010). Treatment with Ag2S2O3 results in the development of stamens in female Silene latifolia (Law et al. 2002), whereas in Cannabis sativa, Cu++ and Zn++
ions along with zeatin accumulation induces feminization effect and Pb$^{++}$ ions along with GA accumulation favors masculinisation effects (Soldatova and Khryanin 2010).

2.2.1.3. Hormonal control

Sex expression in a plant may be influenced and altered by the plant hormones since the reproductive structure is an important part in the life cycle of a plant. Sex regulation in flowering plants is under the influence of various phytohormones such as auxins, cytokinins, gibberellins, and ethylene (Dellaporta and Calderon-Urrea 1993; Dauphin-Guerin et al. 1980; Grant et al. 1994; Irish and Nelson 1989; Jaiswal et al. 1985). Sex expression shows great variability with respect to various plant species under the influence of auxins and cytokinins. Both auxin and cytokinins are considered as a masculalizing as well as feminizing hormones depending upon the species (Chailakhyan 1979; Champault et al. 1981; Dellaporta and Calderon-Urrea 1993; Irish and Nelson 1989; Louis et al. 1990). Auxins show a masculalizing effect on *Mercurialis annua* (Durand and Durand 1984), whereas feminizing effect in *Cannabis sativa* (Galoch 1978). Similarly, the application of gibberellins to female cucumber shows masculalizing effect (Yin and Quinn 1995), whereas application to male flowers in maize gives feminizing effect (Krishnamoorthy and Talukdar 1976). Cytokinins, on the other hand, show a feminizing effect on *Mercurialis annua*, *Vitis vinifera*, *Spinacea oleracea*, and *Cannabis sativa* (Durand and Durand 1984; Chailakhyan and Khryanin 1978; Galoch 1978; Negri and Olmo 1966). Gibberellins show masculalizing effect, whereas ethylene shows the feminization effect (Atal 1959; Hansen et al. 1976; Mohan Ram and Jaiswal 1970; Kumar and Jaiswal 1984).

2.2.2. Regulation of sex expression

Mechanism of sex expression in dioecious plants lacks molecular level investigation, but sexual dimorphism is mainly concerned with floral organogenesis and one or more genes involved in this process. The ABC model is the most accepted model or theory for the development and differentiation of reproductive structures (Coen and Meyerowitz 1991; Weigel and Meyerowitz 1994). The three functional genes (A, B, C) which correspond to the MADS box genes describe how these genes are responsible for the differentiation of floral organs in flowering plants.
The sex expression is usually determined by the genes present on the sex chromosomes or on the autosomes and the suppression of development of one of the sex organs leads to sexual differentiation (Ainsworth 2000; Negrutiu *et al.* 2001; Matsunaga and Kawano 2001). Based on the developmental stage in which plant sex can be detected due to the suppression of one of the sex organs, the dioecious plants are divided into three classes: (1) those whose flower buds rarely form the primordia of the opposite sex *e.g.* Cannabis sativa, Humulus species and Spinacia oleracea (Ainsworth *et al.* 1998; Matsunga and Kawano 2001; Mohan Ram and Nath 1964; Sherry *et al.* 1993); (2) those where development is arrested in the early stages after initiation of opposite sex primordia as in case of Silene latifolia and Pistacia vera (Grant *et al.* 1994; Hormaza and Polito 1996) and (c) those where development is arrested in the later stages *e.g.* Actinidia delicosa and Asparagus officinalis (Caporali *et al.* 1994; Schmid 1978).

A MADS box homolog gene has been identified in two dioecious species. In *Silene latifolia* the expression of five MADS (Minichromosome Maintenance 1, Agamous, Deficiens and Serum response factor) box homolog genes were studied (Hardenack *et al.* 1994). The five genes SLM1, SLM2, SLM3, SLM4 and SLM5 were found to express in both male and female flower meristems, but the expression of SLM2 and SLM3 was more confined to petal and stamens of both male and female, however, it was more pronounced in male flower meristem than female. Concurrently, the reduced gynoecium development was seen in male flower suggesting the role of SLM2 and SLM3 genes in reducing the development of forth whorl in male flowers. Similarly, three MADS box genes RAD1, RAD2 and RAP1 were isolated and characterized in *Rumex acetosa*. RAD1 and RAD2 gene expression was confined to the stamen whorl of a male and female flower. However, the expression of RAP1 was seen in the early stages of stamen and carpel development, but at later stages, when the development of an inappropriate set of organs ceased the expression of RAP1 became undetectable.

### 2.3. SCAR markers and sex typing

SCAR (Sequence Characterized Amplified Region) markers are PCR-based primers that represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification by using sequence specific oligonucleotide primers (Paran and
Michelmore 1993; McDermott et al. 1994). 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 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. Therefore, the plant species used for SCAR development should have sufficient variation in traits of interest at both the DNA sequences and phenotypic levels. Researchers over the years have employed various non-sequence primed PCR markers to develop SCAR markers for the identification of sex in dioecious species of crop plants. Chromosomes and their sex-determining genes have driven a genetic control of sex determination in dioecious crops.

PCR is an in vitro method of nucleic acid synthesis by which a particular segment of DNA can be specifically replicated (Mullis and Faloona 1987). Genomic DNA from two different individuals often produces different amplification products. A particular DNA fragment which represents DNA polymorphism generated from one individual but not from the other can be used as a genetic marker. The pattern of amplified bands obtained could be used as a genomic fingerprint (Welsh and McClelland 1990). The non-sequence PCR-based markers like RAPD, ISSR, and AFLP have been used by various researchers for the determination of sex in dioecious crops, but due to various limitations like reproducibility, repeatability, locus specificity, dominant inheritance, homology in co-migrating amplification products, they cannot be effectively used from laboratory to laboratory because of the difficulty in practical handling, data generation, and analysis and in large scale. An ideal marker should be reliable, simple to generate, and interpret, and it should have sufficient variations of the problem under study. Hence, SCAR markers provide a good platform as they represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification using sequence oligonucleotide primers and arbitrary primed PCR-based markers and they overcome the limitations of non-sequence PCR-based markers. In the recent past, various researchers have focused and developed SCAR markers derived from various nonsequence PCR techniques due to their highly efficient and ideal nature to determine the sex locus in dioecious crops.

Arbitrarily primed PCR-based markers are the markers that require no prior knowledge of the genome that is being analyzed and hence can be employed across
species using universal primers (RAPD, ISSR) or by double digestion of genomic DNA followed by ligation of double-stranded adapters complimentary to the restriction sites and finally selective PCR amplification (AFLP).

2.3.1. RAPD derived SCAR markers in dioecious plants

RAPD analysis combined with bulk segregate analysis has been periodically used for SCAR marker development for sex determination. The first sex-specific SCAR marker was developed by screening 760 decamer primers in dioecious *Asparagus officinalis*. The fragment of decamer primer OPC15-980 which was able to differentiate sexes was converted to SCAR. This SCAR marker developed was able to produce a 980 bp sex-specific band in all male samples (Jiang and Sink 1997). A female specific SCAR marker, using RAPD primer S368 was developed by screening 100 decamer primers. The developed SCAR primers amplified a 928 bp female specific fragment in all female samples. When searched for homologies in the S368bp RAPD fragment, it revealed only limited similarities with repeated regions of retrotransposon-like sequences, suggesting that this sequence might be in the noncoding region of the genome (Gao et al. 2007). Similarly, a decamer primer UBC347 which was able to differentiate the female sex was identified by screening 100 decamer primers and was converted to female specific SCAR marker F400 to be able to amplify a 400 bp fragment in all the female samples of *Asparagus*. Blastx in the NCBI database revealed a 45% sequence identity with a putative retrotransposon protein of rice, belonging to Ty1-copia subclass (Kim et al. 2014). Two SCAR markers SMX and SMY from *Actinidia deliciosa* was developed using sex-differentiating RAPD fragments obtained from RAPD primer OPAI-12 and OPAL-20. The developed SCAR markers showed about 88% success in determining the sex (Gill et al. 1998).

In *Atriplex garrettii*, out of the 158 decamer primers used for screening the sex, only one primer OPAF-14 has revealed a male-specific fragment. This fragment has been isolated, cloned, and sequenced to develop a male-specific SCAR marker SCAR OPAF-14, which later amplified at 2054 bp fragment in all the male plants. Sequence analysis of the fragment has revealed the presence of direct repeats which can be classified into 3 motifs, and a total of 318 bp have been represented in repetitive DNA (Ruas et al. 1998). Over 1040 decamer primers were screened to
identify one decamer primer S1443 which was able to differentiate male and female plants in *Calamus simplicifolius* (Li et al. 2010). Further, the fragment differentiating the sex using this primer has been converted into a SCAR marker CsMale1 that developed a 509 bp fragment in all the male plants. Three SCAR markers namely MADC2 (designed from OPA8400 fragment), SCAR323-MADC5 (designed from OPD05961 fragment), and SCAR119-MADC6 (designed from UBC354151 fragment) that are able to determine the male-specific fragments have been developed in *Cannabis sativa* (Mandolino et al. 1999; Torjek et al. 2002). Sequence analysis of the OPA8400 and UBC354151 fragment showed no or only limited homologies with other plant sequences belonging to repetitive genome regions or retrotransposon elements from different plants such as barley, coconut, pine, pea, and *Arabidopsis*.

In *Carica papaya*, a number of SCAR markers have been developed; Napf (Parasnis et al. 2000) for identification of the male; C09/20 (Niroshini et al. 2008), SCAR SDSP (Chaves-Bedoya and Nunez 2007) and SCARps (Urasaki et al. 2002) for identification of male and hermaphrodite; SCAR T1 (Chaves-Bedoya and Nunez 2007) for identification of female and hermaphrodite and SCAR T12 and SCAR W11 (Deputy et al. 2002) for identification of hermaphrodite individuals. The sequence analysis and blastn search revealed no similarity or compatibility with any known sequence of *C. papaya* in the GenBank database of National Center for Biotechnology Information (NCBI). A SCAR marker SCARmr from female-sex specific fragment MSDE which amplified a 569 bp fragment in all the 10 female plants of *Eucommia ulmoides* has been screened and developed from 560 decamer primers (Xu et al. 2004a). When MSDE fragment was searched for similarities, it showed 53% similarity with the *Arabidopsis thaliana* genomic DNA, chromosome 5 clones: MPH15 in the databases of GenBank and European Molecular Biology Laboratory (EMBL). Male and female specific SCAR markers were developed for *Ginkgo biloba* from RAPD primer S10, which amplifies a 571 bp fragment in females and 688 bp fragments only in males. Further, the sequence showed no homology with any sequence in the NCBI database (Liao et al. 2009). Korekar et al. (2012) developed two female-specific SCAR markers HrX1 and HrX2 in *Hippophae rhamnoides*. This marker amplified fragments of 470 bp and 386 bp in all the female samples (Korekar et al. 2012). Similarly, a male-specific SCAR marker, SDSM in *Momordica dioica* was developed which amplified a 1501 bp fragment in all the male samples (Patil et
al. 2012). When checked for sequence similarities in the 1500 bp fragment isolated from male-specific fragment developed using decamer primer, OPA-15 it revealed similarity with Chromosome11, clone OSJNBa0010E20_PCR of *Oryza sativa* japonica group. SCAR-SDSM sequence showed homology with known ZAT6, MADS box gene SIAP3 of *Silene latilolia*, RAYS1, and RAE 180. In *Mercurialis annua*, the 1562 bp sequence obtained from the male-specific decamer primer OPB-01 was used to design combinations of 7 SCAR primers which specifically amplified 1563 bp, 1553 bp, 1225 bp, 1022 bp, and 1015 bp fragments in male plants (Khadka et al. 2002). In *Pandanus fascicularis*, a male-specific SCAR marker MSSR-01 was developed from RAPD fragment OPD-081263. The designed SCAR marker amplifies a 976 bp fragment in male plants. Sequence analysis using Blastx has revealed that the sequence contains 11 TATA boxes and some ORFs, but it couldn’t code for any proteins (Vinod et al. 2007).

In *Phoenix dactylifera* and *Piper longum*, male specific SCAR markers were developed (Dhawan et al. 2013; Manoj et al. 2005). The sequences of both showed no similarity with any known sequence in the NCBI database. In *Pistacia* species, especially *Pistacia atlantica*, *Pistacia khasjuk*, *Pistacia vera*, and *Pistacia chinensis* female specific SCAR markers (PVP, FS281, FS1 and SCO-08) have been developed where PVP and FS1 amplify a 297 bp, 300 bp, and 636 bp fragments in all female samples (Yakubov et al. 2005; Esfandiyari et al. 2012; Sun et al. 2014) and FS1 and SCO-08 amplify in all male and female samples. Similarly, in *Silene dioica*, *Silene diclinis*, and *Silene latifolia*, 7 male-specific SCAR markers were developed by using RAPD primer OPD-12 (Zhang et al. 1998). In *Salix viminalis*, two female-specific SCAR markers SCAR UBC 354 and SCAR OPAE-08 were designed which could amplify a 354 bp and 780 bp fragments in female plants (Gunter et al. 2003).

No similarities have been noticed within the coding region. Similarities with the sequences are located between the coding regions or exons of putative genes within larger chromosomal or genomic clone fragments of *Arabidopsis thaliana*, *Oryza sativa*, or *Lotus japonicus*. The designed SCAR markers can also amplify successfully in other species of *Salix*. In *Simarouba glauca*, two SCAR markers were developed which amplified a 915 bp fragment in the male and andromonoecious plants (Prasanthi et al. 2010).
2.3.2. ISSR derived SCAR markers in dioecious plants

In *Pseudocalliergon trifarium*, 41 ISSR based universal primers have been screened for identification of sex-specific bands in nine samples. Out of more than 100 scorable bands only one primer, ISSR primer 807 was able to produce a sex-specific band in all the female samples only. This fragment was cloned and sequenced and 3 SCAR primer pairs were designed (PT-1f/PT-1r; PT-1f/PT-2r, and PT-3f/PT-3R). The first primer pair, PT-1f/PT-1r, amplified a 468 bp fragment in all the female samples, but suffered from an uneven quality ranging from strong to weak. The second primer pair PT-1f/PT-2r amplified an 182 bp fragment in both male and female samples. The third primer, PT-3f/PT-3R amplified a 159 bp fragment only in females but not in males. Thus, this primer pair was selected as the female-specific SCAR marker for sex determination in *Pseudocalliergon trifarium* (Korpelainen et al. 2008). In *Piper betle*, 35 ISSR primers were screened for 20 germplasm lines to determine the sex in these plants. Two primers ISSR-10 and UBC-852 produced male-specific bands of 459 bp and 1250 bp only in male samples, whereas only one primer ISSR-23 amplified a 636 bp fragment only in female samples. This female specific band, was isolated, purified, cloned, and sequenced. From the sequence of the female specific band a primer pair SCAR-23 was designed which amplified a 636 bp fragment only in all the female samples (Khadke et al. 2012).

2.3.3. AFLP derived SCAR markers in dioecious plants

Sixty-four AFLP primer combinations along with BSA (Bulk Seggregate Analysis) were used to detect polymorphism and sex-specific bands in *Eucommia ulmoides*. Only one primer combination, E-ACA/M-CTT produced a 350 bp male-specific marker only in all the male samples. This 350 bp band has been isolated, cloned, and sequenced and a pair of forward and reverse primers has been designed. The designed primers amplified a 247 bp fragment in all the male samples, but not in the female samples. The fragment obtained also completely coincided with the corresponding sequence of the AFLP marker. No homology was observed with any sequence of other species in the GenBank (Wang et al. 2011).

In *Ficus fulva*, 3 out of 9 primer combinations screened, developed readily scorable, polymorphic bands. Only one primer combination, Eco-AGC/ Mse-CA produced a fragment of 246 bp which was present in all male samples but not in
female samples. The fragment was sequenced and a male-specific SCAR marker was designed, which amplified a 246 bp fragment in male samples only (Parrish et al. 2004). Out of 24 AFLP primer pairs screened, only one fragment developed from selective AFLP primer pair EA+CC/ MO+CTC showed to be highly intense and present in all males. This fragment was isolated, amplified; cloned and the selected ten clones were sequenced. The seven identified different sequences of *Rumex nivalis* were manually aligned along with the published Y chromosome sequences of *R. acetosa* and used for the development of male-specific SCAR primers in *Rumex nivalis*. PCR amplification of the AFLP derived SCAR resulted in an exclusive male-specific 150 bp fragment in all the male samples. The seven different sequences detected from the sequenced 10 clones of *Rumex nivalis* matched with 67 to 71% identity published Y chromosome sequences of the *R. acetosa* in FASTA database searches (Stehlik and Blattner 2004). In *Bryonia dioica*, one of the six AFLP markers AFLP-278 was used to develop sex-specific SCAR marker. The fragment linked to male sex was isolated and sequenced. The sequences of appropriate length were manually aligned and used to design SCAR primer SCAR-278, which amplified a 278 bp fragment in all the male samples (Oyama et al. 2009).