

Sex-oriented research on dioecious crops of Indian subcontinent: an updated review

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Abstract A number of dioecious species are grown across India and some of those plants play a crucial role in the agro-based economy of the country. The diagnosis of sex is very difficult in the dioecious plant prior flowering wherein sex identification at the seedling stage is of great importance to breeders as well as farmers for crop improvement or production purpose. A comprehensive approach of sex determination comprising morphological, biochemical, cytological and molecular attributes is a must required for gender differentiation in dioecious plant species. In the present review, we highlighted the economical, medicinal as well as industrial importance of most of the dioecious species extensively grown in Indian subcontinent. In addition to that, the cytogenetic, genetic as well as molecular information in

connection to their sex determination were critically discussed in this review.

Keywords Dioecy · Economic importance · India · Medicinal value · Molecular markers · Sex determination

Introduction

Monoecious plants possess independent male and female flowers on the same plant whereas dioecious plants bear male (staminate) and female (pistillate) flowers in different plants. Dioecy is available only in ~7% of flower-bearing plants that cover ~38% plant families (Renner and Ricklefs 1995). Additionally, the occurrence of dioecy is greater among the dicot plants compared to the monocot plants (Ainsworth 2000). It is expected that dioecious species evolved from hermaphrodites or monoecious individuals through two independent mutations resulting in male sterility and reducing female fertility and that ultimately led to functional dioecy (Charlesworth 1991). The most widely accepted hypothesis regarding dioecy evolution is to escape from inbreeding (Barrett 2002). Diverse mechanisms to promote outcrossing include differential maturity of male and female reproductive parts, gametophytic as well as sporophytic self-incompatibility, development of heteromorphic flowers, creation of monoecy and ultimately formation of dioecy. Additionally, it is also expected that the dioecious plants originated from self-compatible species rather than from self-incompatible parents (Barrett 2013).

Generally, three different concepts are available concerning the sex determination in dioecious species viz. hormonal control, epigenetic control and genetic control. Several reports meticulously presented the involvement of diverse phytohormones like gibberellins, ethylene, auxins

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and cytokinins in sex expression of some dioecious plants (Ming et al. 2007, 2011; Milewicz and Sawicki 2012). In some dioecious plants like *Silene latifolia*, *Melandrium album* and *Cannabis sativa*, epigenetic regulation of sex-determination was reported (Law et al. 2002; Soldatova and Khryanin 2010; Milewicz and Sawicki 2012). Several studies were carried out for considering the genetic control of sex determination in dioecious plants. The genetic control of sex determination may be single locus or multiple loci controlled. In case of multiple loci governed sex determination system chromosome constituents may be either homogametic or heterogametic in nature. In *Atriplex garrettii* and *Ecballium elaterium* (squirting cucumber), a single locus determines the sex of the plants whereas in *Mercurialis annua*, sex is controlled by multiple unlinked loci (Heikrujam et al. 2015). In several dioecious species including white campion (*S. latifolia*), asparagus (*Asparagus officinalis* L.), date palm (*Phoenix dactylifera* L.), palmyra palm (*Borassus flabellifer* L.), pointed gourd (*Trichosanthes dioica* Roxb.), teasle gourd, ivy gourd (*Coccinia indica* Wight and Arn. and *C. grandis* (L.) Voigt), kiwifruit (*Actinidia deliciosa* var. *deliciosa*) and hemp (*C. sativa* L.) homogametic female (XX) and heterogametic male (XY) plants were documented (Ming et al. 2007; Cherif et al. 2013) but, a recent study also reported supermales (YY) in asparagus (Harkess et al. 2015). In papaya (*Carica papaya*), which is trioecious in nature due to availability of three sex types (male, female and hermaphrodite), XY system is available and two distinct Y chromosomes viz. Y and Y^h were reported. Y chromosome determines the development of male flowers whereas Y^h chromosome, which is 1.2% diverse than the Y chromosome at DNA level, governs hermaphrodite flower development (Wang et al. 2012). In contrast to the XX female and XY male plants mentioned earlier, certain dioecious species like pistachio, yam, nutmeg, wild strawberry (*Fragaria elatior*) and willow (*Salix viminalis*) were found to have heterogametic female (ZW) and homogametic male (ZZ) chromosomes (Hormaza et al. 1994; Vyskot and Hobza 2004; Kafkas et al. 2015). In some dioecious species like sorrel (*Rumex acetosa*) and hops (*Humulus japonicas*, *H. lupulus*) not only the sex chromosome but the balance between the sex chromosome and autosome (X:A) governs the sex of the plant (Ainsworth et al. 2005; Shephard et al. 1999, 2000). Under that category, female sex possesses X:A ratio greater or equal to 1.0 but for male sex, the X:A ratio is lesser or equal to 0.5.

Different modes of sex chromosome evolution and their modifications were documented by several researchers (Vyskot and Hobza 2004; Ming et al. 2011) and Fig. 1 depicts the fact that how sex chromosomes of plants were evolved. Although several researches were carried out to unravel the genetic mystery regarding the sex

determination in plants, there is very little genomic information available for dioecious species. To our knowledge, among the major dioecious species the genetic maps for the sex chromosome are available only in case of *H. lupulus*, asparagus and kiwifruit (Telgmann-Rauber et al. 2007; Seefelder et al. 2000; Fraser et al. 2009), while sex-linked genes were mapped in *Silene* species (Bergero et al. 2013). A study in *Rumex hastatulus* revealed the distribution of cytonuclear interacting genes (genes from nucleus, mitochondria and chloroplast) with sex chromosomes (Hough et al. 2014). A recent review aptly presented several discoveries regarding the sex determination in dioecious plants at genetic and chromosomal level and updated about different sex-specific genes in monoecious as well as dioecious plants (Kumar et al. 2014). Further research is needed to understand the molecular basis of sex determination in model as well as cultivated dioecious plants grown worldwide. In this present review, we focused on the economically important dioecious plant species grown in India, their medicinal or commercial utilities as well as several key studies regarding their sex determination. The names of those dioecious species (under discussion) have been presented in Table 1 along with the major Indian states producing those crops.

Pointed gourd (*Trichosanthes dioica* Roxb.)

Pointed gourd (*T. dioica* Roxb.), commonly known as *parwal* (in India), belongs to the family Cucurbitaceae. It is a perennial crop extensively grown in tropical and subtropical parts of the world. It holds a stable position at Indian vegetable market for more than 8 months including *kharif* and *rabi* seasons (Banu et al. 2007; Shivhare et al. 2010). It is also called as 'green potato' or the 'king of gourd' because of its wider acceptance as vegetables with nutritive values. Vitamin C content of pointed gourd is much higher than that of any other gourd. The pointed gourd has numerous medicinal values in its fruit, stem and leaves. The leaves have blood sugar improvement properties (Rai et al. 2008) and the juice extracted from the leaf is used to treat alopecia areata affected patches (Prajapathi et al. 2003). The fruit is diuretic in nature and used for improving appetite as well as digestion and in addition to that it possesses cardiotoxic, antiulcerous and anthelmintic properties. The fruit can also reduce blood sugar and serum triglycerides levels in the body and can be used against spermatorrhoea and skin diseases (Sharma et al. 1990). The tender shoots and juice of unripe fruits are used as laxatives. The fruits as well as seeds have some anticancer and haemagglutinating activities while the seeds are reported to have antidiabetic activity (Rai et al. 2010). The decoction is useful in skin diseases and used as a febrifuge. Another

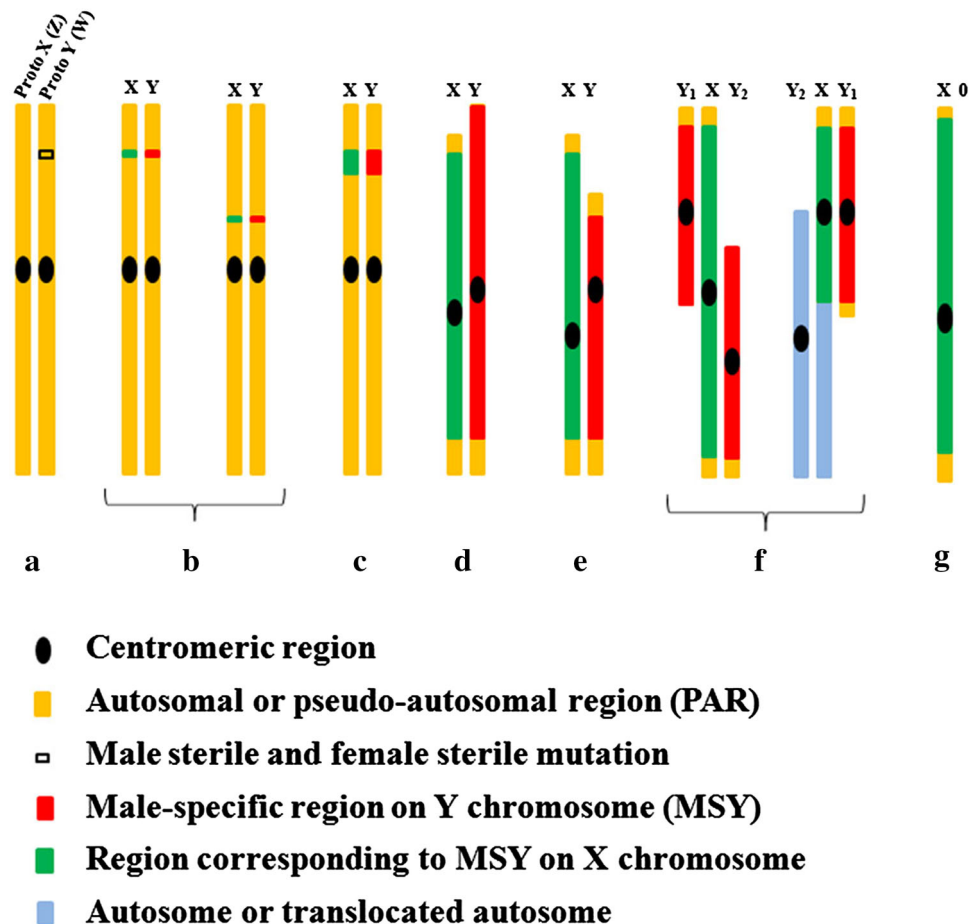


Fig. 1 A schematic diagram of plant sex chromosome evolution that confirms the pivotal role of Y chromosome for the maleness in dioecious plant species. It can be illustrated as follows: **a** in the XY or ZW system, mutation causing functional loss in one chromosome and subsequently conferring complementary dominance to its homologue. As a result, either male or female sterility is induced. Here, recombination among the sex-determining loci produces many hermaphrodites and neuters also; **b** a small male-specific region on Y chromosome (MSY) is developed as a result of suppression of recombination between the sex-determining loci. As a result, the YY males can be found; **c** here the MSY continues to expand due to duplication of Y-linked genes or accumulation translocation products

and transposons causing loss of many genes; therefore, YY genotypes cannot be found in the population; **d** in this case the MSY occupies majority of the Y chromosome causing elongation of Y chromosome than the X homologue, i.e. they become heteromorphic; **e** shrinkage of Y chromosome occurs due to deletion of many nonfunctional genes; **f** due to translocation of autosomal segment into the X chromosome and its co-segregation with the normal Y homologue afterwards generates a multiple Y chromosome system (XY₁Y₂); **g** the entire Y chromosome suffers no recombination causing complete loss of Y from the genome and X-to-autosome ratio for sex determination system arises

study in rats reported that the fruit extract of pointed gourd possesses wound healing and cholesterol-lowering activity (Banu et al. 2007; Shivhare et al. 2010).

Owing to the poor seed viability and germinability, vine and root cutting are recommended for commercial propagation. The major insect pollinator of pointed gourd is red brown beetle (*Carpophilus dimidiatus*), but in India, hand pollination is done for better fruit set in this dioecious crop (Hazra et al. 2011). The chromosome number of pointed gourd was reported to be $2n = 22$ from both sex without having any distinct sex chromosome (Sinha et al. 2007). A noticeable proportion for female plants over males (2.4:1) was observed by Kumar et al. (2008) by in vitro culture of

almost fully developed embryos; the probable cause might be due to the action of lethal/sub-lethal gene(s) linkage with the female sex confirming locus. Hazra et al. (2011) reported a pair of physiologically distinct chromosomes carrying the sex determining genes in pointed gourd, whereas later on, Karmakar et al. (2013) karyotyped another dioecious *Trichosanthes* (*T. bracteata*) revealing the absence of XY mechanism.

During the last one and half decade, a number of researches were carried out employing the molecular marker-based sex determination in *Trichosanthes* species. Although Singh et al. (2002), for the first time, reported a female-specific 567 base pair (bp) amplicon using randomly amplified polymorphic

Table 1 Economically important dioecious crops grown in different states of India

| Dioecious plants | Major growing states | Major economic importance in India |
|-----------------------------|---|------------------------------------|
| <i>Trichosanthes dioica</i> | West Bengal, Assam, Tripura, Arunachal Pradesh, Orissa, Jharkhand and Bihar | Used as vegetable |
| <i>Coccinia</i> species | West Bengal, Tripura and Assam | Used as vegetable |
| <i>Momordica</i> species | West Bengal, Assam and Tamil Nadu | Used as vegetable |
| Asparagus | Himachal Pradesh | Used as vegetable |
| Betel vine | West Bengal, Andhra Pradesh, Kerala, Assam, Tamil Nadu and Karnataka | Used as masticatory |
| Long pepper | Kerala and Andhra Pradesh | Used as spice |
| Nutmeg | Kerala, Tamil Nadu and Karnataka | Used as spice |
| Pistachio | Jammu & Kashmir and Himachal Pradesh | Used as condiment |
| Date palm | Rajasthan, Gujarat and Punjab | Used as fruit |
| Palmira palm | Kerala | Used as fruit |
| Rattan palm | Assam and Arunachal Pradesh | Used to make furniture |
| Mulberry | Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir | Used in silk industry |
| Kiwifruit | Himachal Pradesh | Used as fruit |
| <i>Pandanus</i> species | Orissa and West Bengal | Used as condiment |
| Jjoba | Rajasthan | Used in oil and cosmetic industry |
| Yam | Orissa | Used as starchy food |
| Hemp ^a | – | – |

^a Cultivation of hemp in India is prohibited by law

DNA (RAPD) primer (OPC07), the same primer failed to reproduce that particular band in a local popular variety (Kajli) of *T. dioica* in our experiment (unpublished data). Hence, an uncertainty remains on using OPC07 to screen the female plants of pointed gourd. Further studies identified three polymorphic RAPD markers for sex determination in pointed gourd viz. OPC05 amplified 1000 bp for male plant, OPC14 amplified 400 bp for female plant and another one (OPN01) showed 1030 bp amplification in both male and female plants but did not produce any band in the parthenocarpic plants (Kumar et al. 2012). From one inter-simple sequence repeat (ISSR) marker, Nanda et al. (2013) resized a sequence-tagged site (STS) marker (TdSTSM) that could amplify 720 bp amplicon in the male flower bearing plants only. Additionally, the amplified region was used in Southern blotting to confirm the single copy male-specific locus in pointed gourd. Recently, another group of scientists screened 42 ISSR primers to reveal the sex differentiation in pointed gourd (Adhikari et al. 2014); out of these primers, only ISSR-6 with the sequence (GACA)₄ showed ~500 bp amplification distinctly in male plants.

Ivy gourd (*Coccinia* species)

Ivy gourd (*C. indica* Wight and Arn. and *C. grandis* (L.) Voigt), another important member of Cucurbitaceae, is also known as *kundru* (in India). It is a rapidly growing perennial climber which is vegetatively propagated and widely cultivated in India. The whole plant of *C. grandis*

was found to possess numerous pharmacological activities including antibacterial, hepatoprotective, antitussive, antioxidant, hypoglycaemic, antimalarial, antihelminthic, anticancerous, antidyslipidemic, antiinflammatory, analgesic, antipyretic, alpha amylase inhibitory activity, etc. (Tamilselvan et al. 2011; Hussain et al. 2011; Pekamwar et al. 2013). Ivy gourd is rich in β -carotene and traditionally it is widely used in folklore medicine against diabetes, ulcers, stomach ache, skin disease, fever, cough, asthma, jaundice, wound healing, anaemia and gonorrhoea (Hussain et al. 2011). Following some literatures, the somatic chromosome number may be $2n = 24, 36$ or 48 (Guha et al. 2014; Ghadge et al. 2014) while according to other reports the male plants are heterogametic with $22 + XY$ and the female are homogametic with $22 + XX$ (Sinha et al. 2007; Sousa et al. 2013). According to Guha et al. (2014), the Y chromosome is noticeably 3–4 times larger than almost all of the other chromosomes but karyotyping of *C. grandis* revealed the size of the Y chromosome twice of any other chromosomes and its complete heterochromatic nature was documented by C-banding (Sousa et al. 2013). Additionally, fluorescence in situ hybridization (FISH) staining revealed the absence of 5S and 45S rDNA sites on the Y chromosome and genomic in situ hybridization (GISH) using various femaleness blocking DNA concentrations, discovered the possibility of the presence of pseudo-autosomal region on it (Sousa et al. 2013). According to Ghadge et al. (2014), Y chromosome possesses the pollen fertility factor as well as the region containing ‘female suppressing’ functions in *C. grandis*.

Very little work has been performed on *Coccinia* species regarding the identification of sex-related molecular marker. In a study conducted by Bhowmick et al. (2014), a male-specific RAPD marker (designated as CgMMSM) was identified in *C. grandis*. Out of nineteen RAPD primers tested, only the primer OPC08 produced a male-specific amplification of ~830 bp. One sequence characterized amplified region (SCAR) marker (CgY1) with 829 bp amplification was also developed in that study which was available in male plants only.

Momordica species

There are four dioecious species under the genera *Momordica* and all of them are from the family Cucurbitaceae; the first three viz. *M. dioica* Roxb., *M. sahyadrica*, and *M. cochinchinensis* (Lour.) Spreng. have $2n = 28$ and the fourth one i.e. *M. subangulata* Blume subsp. *renigera* (G. Don) has $2n = 56$ (Bharathi et al. 2011) chromosomes. They are perennial creepers and propagated vegetatively through tuberous roots. Due to the presence of deep red colour in the aril, i.e. seed pulp, for the availability of considerably more carotenoid, the ripe gac (*M. cochinchinensis*) fruit is massively used to colour the rice grains by the Vietnamese (Aoki et al. 2002; Vuong et al. 2006). Zheng et al. (2014) proved the anticancerous property of gac seeds. In India, sweet gourd or gac fruit is an underutilized vegetable but the other three are popularly known as *kantola* or spine gourd or teasle gourd and fruit; young twigs as well as leaves are widely consumed as vegetables.

The *M. dioica* has several medicinal uses (Talukdar and Hossain 2014). Dried fruit powder produces a powerful errhine effect when applied into the nostrils. Fresh fruit juice is consumed against hypertension and diabetes and extract from the leaves is applied as ointment to relieve headache while the leaf paste can be used against the eczema and other skin problems. To treat snakebite and scorpion sting, the root of the male plant is used while the tuber of female plant and toasted root are used to treat bleeding piles and bowel infections. The tubers of the teasle gourd act as antihelminthic, spermicidal, and antifertility as well as abortifacient agent. Beside these, it has antioxidant, analgesic, nephroprotective, neuroprotective, antiallergic, antiulcer, anticancer, antimicrobial, anti-malarial, hepatoprotective, antihepatotoxic, antiedemic and insecticidal properties (Talukdar and Hossain 2014). Narasimhan et al. (2005) showed antifeedant activity of *M. dioica* against *Spodoptera litura* and the seed oil of *M. dioica* could be used as grain protectant against *Callosobruchus chinensis* (Mishra et al. 2006). Later on, Ahire and Deokule (2012) showed the allelopathic activity of *M. dioica* on *Phaseolus aconitifolius* Jacq.

An attempt was made for the first time to understand the genotypic differentiation between male and female plants of *M. dioica* at molecular level using RAPD primers (Baratakke and Patil 2009). Out of 20 RAPD primers tested, only OPA-15 showed unique male-specific 1625 bp amplification. Subsequently, the same group screened 50 RAPD primers and among them only OPA-15 showed male-specific amplification as detected earlier but the amplicon size was ~1500 bp. Using the sequence information of the male-specific 1500 bp band, two SCAR primers viz. MSSM-01F and MSSM-01R were developed which could specifically amplify 1501 bp DNA only from the male plants but not from the female plants (Patil et al. 2012).

Garden asparagus (*Asparagus officinalis* L.)

Garden asparagus (*A. officinalis* L.), a plant from the Asparagaceae family, is herbaceous perennial plant and it is propagated through seeds. The tender succulent shoots are eaten as vegetable that is nutritious and rich in vitamins. The tender shoots or spears are cooked as vegetable, soup and it can be eaten raw (Kabir 1993). Although they are dioecious but 1–2% plant are andromonoecious carrying hermaphrodite and male flowers on the same plant (Telgmann-Rauber et al. 2007). The male flowers are yellowish green and conspicuous, while the female flowers are less conspicuous. Male asparagus plants are comparatively more disease resistant, have better yields and live longer than female plants (Kabir 1993) and also they do not produce seeds to create ‘asparagus weed problem’. According to Harkess et al. (2015), the chromosome number of diploid asparagus is $2n = 20$ with $x = 10$. The females, males and supermales have XX, XY and YY sex chromosomes, respectively, and specifically males as well as supermales possess genes involved in pollen microspore and tapetum development.

As asparagus has a global market and male plants are preferred compared to the female plants by the growers, several studies were carried out for identifying different sex-specific molecular markers in this crop. A total of 760 RAPD primers were used by Jiang and Sink (1997) to explore the male sex locus *M* of asparagus using a mapping population and only one primer (OPC15) showed two polymorphisms (OPC15-98 and OPC15-30) specific to sex locus *M*. Using the sequence information of OPC15-98 marker, an SCAR primer was developed which could amplify 980 bp specific region from the male plants only. Interestingly, the SCAR marker was not found suitable for other asparagus germplasm studied by the same group (Jiang and Sink 1997). In the following year, another group identified amplified fragment length polymorphism (AFLP)

marker E41M50 providing strong male-specific linkage in *A. officinalis* (Reamon-Büttner et al. 1998). Later on, nine AFLP markers (showing sex linkage) and two STS markers were used to identify 13 different bacterial artificial chromosome (BAC) clones generated from one male plant carrying the male flowering gene at homozygous state (*MM*) (Jamsari et al. 2004). Using the Asp1-T7 marker, previously used by Jamsari et al. (2004), a PCR based marker pair (Asp1-T7spf and Asp1-T7spr) was generated, which could produce 308 bp amplicon specific for maleness (Nakayama et al. 2006). Using the RAPD primer S368, Gao et al. (2007) identified two different female-specific markers (S368-928 and S368-1178). Between these two markers, S368-928 was found to be linked with the female sex locus and using the sequence information an SCAR marker was developed to identify homozygous female (*mm*) plant. Interestingly, Southern blot analysis revealed that the genomic region corresponding to S368-928 was found to be available in both the sexes (male and female). Another RAPD primer (T35R54) amplified 1600 bp male-specific region between the tested supermale and normal male plants (Ii et al. 2012). Kanno et al. (2014) derived a male-specific STS from the T35R54-1600 and it was identified as another sex-linked region. The identified primer pair (MSSTS710-fw and MSSTS710-rv) amplified 710 bp male-specific amplicon in eight different cultivars of *A. officinalis*. Although the earlier reported Asp1T7sp marker (Nakayama et al. 2006) was able to generate male-specific amplifications in two different species of asparagus, namely *A. schoberioides* and *A. kiusianus*, MSSTS710 failed to show any amplification in those two species (Kanno et al. 2014).

Betel vine (*Piper betle* L.)

This is a cash crop in India (commonly known as *paan*) from the family Piperaceae. It is a perennial and vegetatively propagated evergreen creeper favouring tropical forest climate having shade, high humidity and warm temperature. It has a wide range of somatic chromosome number ($2n = 45, 48, 52, 56, 64, 72$) and according to Samantaray et al. (2012), males are triploids ($3x = 39$) whereas females are tetraploids ($4x = 52$). Leaves of female plants are mostly pungent and are cordate or ovate to round in shape whereas in case of male plants the leaves are non-pungent and are narrowly ovate. Two types of branches are produced in betel vine, namely orthotropic (vegetative) and plageotropic (reproductive). The male and female spikes are long and short, respectively, and those arise singly from the leaf base (Maiti 2006).

Various compounds, namely chavicol, hydroxyl-chavicol, chavibetol and chavibetol acetate were identified from

betel leaves possessing medicinal values to cure digestive problems and were used for healing wounds, burns, gum sores as well as to prevent cancer (Rai et al. 2011). Betel leaves are also utilized in various Hindu religious purposes and it has great economic importance as it is taken as regular post-meal item masticated by many Indian people.

Since the last decade, researches have carried out sex-specific molecular marker identification in this important commercial horticultural crop of Southeast Asia. Different RAPD primers, especially OPB-08, OPF-09, OPF-07 and OPG-16, showed differential banding pattern among the male (*Desavari*) and female (*Bangla*) plants of *P. betle* (Usha et al. 2009). Another group identified 1400 and 850 bp male-specific amplification using OPA04 and OPN02 RAPD primers, respectively, and 980 bp female-specific amplifications using OPC06 primer; additionally PCR amplification using RAPD primer OPA08 generated 650 bp amplicon only in male DNA samples and 1200 bp PCR product only in female DNA samples (Samantaray et al. 2012). Further researches identified two amplicons viz. 459 and 1250 bp, specific for maleness, using ISSR primers ISSR-10 [(GA)₆CC] and UBC-852 [(TC)₈RA], respectively and a female-specific amplification of 636 bp fragment using ISSR-23 [(GACA)₃CC] primer (Khadke et al. 2012). Using the female-specific amplicon produced by ISSR-23 primer, a successful attempt was made to develop a SCAR marker (primers designated as IS-23 F/R) that could amplify a 636 bp band only in female plants but not in males. Another group developed a SCAR marker derived from a RAPD marker for the first time in betel vine (Sheeja et al. 2013). Out of 82 RAPD primers, primer OPE-11 obtained a male-specific amplification of about 600 bp whereas the primers OPE-1 and OPB-20 showed female-specific amplifications of about 400 and 488 bp, respectively. A female-specific SCAR marker (primer used: Pibet-20 F and Pibet-20 R) having the accession number JN228255 was developed using the 488 bp female-specific amplicon obtained by the primer OPB-20 (Sheeja et al. 2013).

Long pepper (*Piper longum* L.)

Long pepper (*Piper longum* L.), popularly known as *Pipal* in India, belongs to the family Piperaceae. It is a slender aromatic perennial climber and is well known for its medicinal property. The plant is propagated through seeds, suckers or cuttings. The somatic chromosome number is $2n = 24, 48, 52, 96$ (Farooqi et al. 2005). Female spikes are shorter and thicker than the male spikes and mature female spikes are extensively used against respiratory tract diseases traditionally in Indian Ayurveda (Viswanathan 1995). The dried fruit is used as a spice, while the roots or

Piplamul are used both in ayurvedic and Unani systems of medicine. The root and fruit are used in palsy, gout and lumbago whereas the roots are used to cure abdominal problems, spleen diseases and tumour also (Farooqi et al. 2005).

The first attempt to identify sex-specific molecular marker in *P. longum* was carried out by Banerjee et al. (1999) using RAPD primers and among the tested primers OPA-10 and OPAC-12 showed male-specific amplification of around 908 and 757 bp, respectively. Further studies identified two other male sex-associated amplifications of around 827 and 744 bp using RAPD primers OPA10 and OPA15, respectively (Manoj et al. 2005). Furthermore, using these amplifications two SCAR markers were developed and the amplicons are available in the GenBank, NCBI having accession numbers AY046931 and AF375880. The pair of SCAR markers (MPS1A and MPS1B) could amplify the sequence MADP1 (male-specific DNA) having accession number AY046931 specifically from the male-specific DNA samples only, whereas the SCAR marker based on MADP2 sequence showed 744 and 747 bp amplifications from male and some of the female plants, respectively (Manoj et al. 2005). Moreover, the same group identified several sex-specific molecular markers (3 male-specific and 8 female-specific) by polymerase chain reaction (PCR) based approach.

Nutmeg (*Myristica fragrans* Houtt.)

Nutmeg tree (*Myristica fragrans* Houtt.) or *jaephal* (common name in India) comes under the family Myristicaceae and produces two separate spices from the fruit, namely nutmeg and mace; the dried seed kernel is called nutmeg whereas mace is the dried aril covering the single seed. It is a spreading evergreen tree with somatic chromosome number $2n = 42$ and can be propagated vegetatively mainly by grafting or from seeds (Farooqi et al. 2005). It has been suspected that a female heterogametic system (ZZ male and ZW female) exists in nutmeg (Heikrujam et al. 2015). The male has smaller and fewer leaves than the female. Although nutmeg and mace are largely used as flavouring agent, several studies reported the potential utility of nutmeg as antibacterial, antiviral, antidiabetic, and antileukaemic agents (Farooqi et al. 2005; Latha et al. 2005; Yang et al. 2006; Chirathaworn et al. 2007).

Almost 6–8 years is needed to differentiate the sex in *M. fragrans*. The female plants are economically productive and early detection of sex is needed to reduce the male plants about up to 50% of the population. Till date to our knowledge only a single report is available regarding the availability of sex-associated molecular marker in this tree

species. Out of 60 different RAPD primers tested, only the primer OPE 11 produced a female-specific ~416 bp amplification in nutmeg, but the same band was not detected in male samples (Shibu et al. 2000).

Pistachio (*Pistacia vera* L.)

Pistachio (*Pistacia vera* L.) or *pista* (common name in India) is a small deciduous tree belonging to the family Anacardiaceae and it has $2n = 30$. Flowers of pistachio are apetalous and wind is the pollinating agent for the plant. The edible portion of it is the seed kernel that can be used as an ingredient of sweet meats, confectionery and bakery industries. Along with the propagation through seeds, pistachio can be vegetatively propagated through budding and grafting (Thakur and Rathore 1991). The sex chromosome of pistachio was identified by meiotic study (Harandi and Ghaffari 2001) whereas karyotyping revealed heteromorphic (XY) sex chromosomes in male pistachio plants (Tilkat et al. 2011).

In the last decade of twentieth century, an attempt was made to identify sex-specific molecular marker in *P. vera* using 700 different primers comprising of oligonucleotide decamer. Out of these tested RAPD primers, only one primer (OPO08) produced a 945 bp fragment and the band was only found from the female samples (Hormaza et al. 1994). Another study found sex-specific amplification using nine different ISSR markers and out of them only two primers, (AC)₈CG and (AC)₈TA, were able to discriminate between the female and male plants of *P. vera* showing some female-specific amplifications (Ehsanpour et al. 2008). Molecular marker based sex determination approach was taken for different pistachio species (*P. atlantica* Desf. subsp. *mutica*, *P. khinjuk*, and *P. vera* subsp. *sarakhs*) using thirty different RAPD primers and out of them only BC1200 showed a female-specific amplification at around 1200 bp (Esfandiaryari et al. 2011). Additionally, an SCAR marker was identified in that study which was able to amplify a 300 bp region from the female plant only. Very recently, using sex-specific single-nucleotide polymorphism (SNP) markers and restriction site associated DNA (RAD) sequencing technique, Kafkas et al. (2015) identified eight loci regarding sex differentiation in pistachio; finally, they identified four perfect sex distinguishing markers by high-resolution melting (HRM) analysis.

Date palm (*Phoenix dactylifera* L.)

Date palm (*P. dactylifera* L.) or *khajur* (common name in India) is from the family Areaceae/Palmae/Palmaceae and since ancient time it is cultivated as fruit tree and known as

'Tree of Life' by the ancient people (Nath et al. 2008). It has $2n = 36$ and being dioecious the commercially important date fruits are only borne by the female plants. Six- to eight-year-old trees start flowering when sex differentiation can be done visually (Mathew et al. 2014). Cherif et al. (2013) found a non-recombining region in the date palm genome confirming the presence of XY system. Pollination occurs by wind and insects but for better fruit set artificial pollination is done. Crude sugar, molasses, alcoholic beverages are prepared from delicious sweet sap found by tapping date palm tree. Commercially date fruits are used to prepare highly nutritive fruit products; the leaves are used to prepare many things like baskets, brooms, ropes, etc. and the dried leaves as well as trunks are used as fuel also. Although the date fruit is marketed as confectionery all over the world, the demand for fresh fruit especially in the deserts remains high. The fruit is a rich source of tannin which is antiinfective, antiinflammatory and antihemorrhagic, and along with that many other antioxidants like beta-carotene, lutein and zeaxanthin are also found in date fruit that can prevent cancer. Additionally, the dietary fibre present in date fruit can prevent LDL cholesterol absorption in the gut (Dada et al. 2012).

Different researchers identified a number of RAPD primers for sex identification in date palm. One RAPD primer amplified two unique alleles of 600 and 750 bp for differentiating the male and female plants, respectively (Singh et al. 2006), whereas another group was able to identify five sex-specific (3 for female and 2 for male) markers using three different RAPD primers (Younis et al. 2008). Additionally, Younis et al. (2008) also reported five different male-specific amplification (of about 340, 1010, 375, 590 and 920 bp long) using five different ISSR primers. Another attempt was made to identify sex-specific microsatellite markers from date palm and 14 simple sequence repeat (SSR) primer pairs were used to screen 34 cultivars of date palm (Elmeer and Mattat 2012). Out of 254 numbers of detected microsatellite loci, 22 were found to identify 75% male samples of date palm. Later on, Dhawan et al. (2013) screened 100 RAPD primers and 104 ISSR primers for sex determination in date palm and identified single RAPD (OPA-02) primer amplifying about 1.0 kb DNA band only from the DNA samples of the male date palms. By cloning and sequencing of that RAPD band, they designed SCAR primer pair for assured sex differentiation of date palm which amplified a 406 bp amplicon in both male and female plants and a 354 bp fragment in males only. Another group reported two different approaches regarding date palm sex discrimination (Al-Mahmoud et al. 2012). In the first approach, they used PCR-based RFLP analysis and in the second approach they did only PCR using the availability of high heterogeneity in the sex-linked region and no restriction digestion was performed in

that approach. Although they found different polymorphisms among the male and female date palms at their early growth stages using both of these approaches, the PCR-RFLP approach was more reliable compared to the PCR only method. Recently, start codon targeted polymorphism (SCoT) and RAPD analyses revealed two SCoT primers (SCoT36 and SCoT41) and four RAPD primers (OP-A11, OP-M11, OP-O07 and OP-S07) showing differential sex-specific banding pattern in date palm (Sami et al. 2014). Two SCoT primers, SCoT41 and SCoT36, identified single female-specific amplification for each of them at around 1150 and 850 bp, respectively whereas four different RAPD primers exhibited three female-specific and two male-specific amplifications of different sizes (Sami et al. 2014).

Palmyra palm (*Borassus flabellifer* L.)

Palmyra palm (*B. flabellifer* L.) or *taar* (common name in India) is an underexploited tree belonging to the family Arecaceae and usually found growing almost wild in every type of wasteland. It is propagated from seeds. It is allotetraploid and the somatic chromosome number is $2n = 36$ ($x = 8$ or 9). The male and female have XY and XX pair of sex chromosomes, respectively. The male spadix contains 20,000–25,000 florets while the female spadix has 30–75 flowers. The pollens are borne by insects as well as wind and the fertilized flower takes 120–130 days to become matured fruit (Chattopadhyay and Bose 2006). A delicious sugary sap is obtained on tapping both the male or female spadices which can be fermented into *toddy* (country liquor) or boiled down into jaggery, sugar, molasses, vinegar, etc. The edible parts are the endosperm of the tender fruit and the mesocarp (pulp) of the ripe fruit. Tapping sap and the tender fruit endosperm are natural coolants, while the fleshy roots and the young bud present inside the tree apex are also eaten as a diuretic. Additionally, the leaves are used for thatching purposes and the leaves as well as fibres are utilized for various handicraft preparations (Chattopadhyay and Bose 2006; Thiruchitrambalam and Shanmugam 2012) while the trunk is used as wood, especially for making poles. Due to diverse utilization of the whole plant, the palmyra palm is popularly known as 'Tree of Life'. In spite of the importance of this plant, the identification of the sex of this plant is very problematic at early growth stages. To escape the long waiting period (12–15 years) for identification of the sex of palmyra palm, very little work has been carried out using molecular markers. One male-specific RAPD primer was identified and it was found to produce a polymorphic DNA band of ~600 bp in the male plant (George et al. 2007). Further study by the same group was carried out for

early determination of sex in one, two and three-seeded fruits of *B. flabellifer* L. using the same primer as before (George and Karun 2011).

Rattan palm

Rattan palm or *Calamus* species, belonging to the family Palmae, is another important dioecious plant predominantly grown in tropics and subtropics. Next to bamboo, rattans are spiny palms producing important non-wood forest products worldwide (Xu et al. 2000) and bear typical scaly fruits (Sarmah and Sarma 2011). Rattans are acknowledged as 'green gold' for their valuable economic characters like appearance, lightness, strength, flexibility as well as durability (Mohan and Tandon 1997). There is no record regarding sex chromosomes in *Calamus* genus. They have usually chromosome numbers $2n = 2x = 26$ (Sarkar and Datta 1985; Roser 1994) but *C. palustris* Griff. with $2n = 2x = 28$ is an exception (Indira and Anto 2002). *C. simplicifolius* has worldwide acceptance for high-quality canes suitable for furniture and handicraft making and also for its shoots which is consumed as vegetable (Li et al. 2010). Among different species of rattan palm, *C. tenuis* Roxb. is predominant in Indian subcontinent (Sarmah and Sarma 2011).

A few groups of researchers worked on the molecular markers for sex identification in *Calamus* species. A male-specific RAPD primer (S1443), producing 500 bp fragment, was developed by Yang et al. (2005) in *C. simplicifolius*. In the same species, utilizing an RAPD primer, Li et al. (2010) constructed a male-specific SCAR marker (CsMale1) producing 509 bp amplicon and that possibly represented a coding genomic region due to the presence of two open reading frames in that sequence. Another group identified an ISSR primer with the sequence (AAG)₅ CC and that could amplify 600 bp fragment in female *C. tenuis* Roxb. only but not in the male one (Sarmah and Sarma 2011).

Mulberry (*Morus* spp.)

There are four important species of mulberry: *Morus alba* L. (white mulberry), *M. rubra* L. (red mulberry), *M. nigra* L. (black mulberry) and *M. microphylla* Buckl. (little-leaf mulberry) and they belong to the family Moraceae. Generally, mulberry plants are dioecious in nature, but some monoecious branches can be present in a plant (Barbour et al. 2008). Bhattacharya and Ranade (2001) reported several physiological and biochemical factors causing different sex types of mulberry viz. female, male as well as hermaphrodite flowers bearing monoecious type or

dioecious type. Both white and red mulberry plants have $2n = 2x = 28$ chromosome constitution, but black type has $2n = 22x = 308$ (Ottman 1987). In India, mulberry is economically important to rear the silkworm (*Bombyx mori* L.) larvae that feed on the leaves. Mulberry wood can be utilized for making furniture and sport items while paper can be made from the bark of the tree. The ripe fruit is also taken as delicious fruit that can cure throat infection, purify blood and control fever. It has been reported that nerve tonic can be prepared from the bark of mulberry (Nath et al. 2008).

Very little work has been done for identifying the sex of mulberry by molecular means. A study on diversification of mulberry using ISSR and RAPD molecular markers identified an ISSR marker (ISSR-825.450) having the sequence (AC)₈T associated with the female sex only, whereas an RAPD marker (OPY-15.1200) showed about 1200 bp amplification only in male plants (Vijayan et al. 2009).

Kiwifruit (*Actinidia deliciosa* var. *deliciosa*)

Chinese gooseberry or kiwifruit (*Actinidia deliciosa* var. *deliciosa*) comes under the family Actinidiaceae and it is also called China's miracle fruit and Horticultural wonder of New Zealand. It is a perennial vine and can be commercially propagated by softwood cutting as well as from the seed (Rathore 1991). In India, kiwifruit was introduced in 1960s in parts of the Himalayan region where it performed successfully (Dadlani et al. 1971). There are 60 species of the genus *Actinidia* but *A. deliciosa* var. *deliciosa* and *A. chinensis* are the only two species bearing edible fruits. Furthermore, *A. deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson, commonly known as kiwifruit, is the only species cultivated all over the world (Sharma and Shirkot 2004). The diploid ($2n = 58$) species *A. chinensis* is the progenitor of *A. deliciosa* var. *deliciosa* ($2n = 6x = 174$). XY-type system exists in *Actinidia* with heterogametic male (Harvey et al. 1997); the sex chromosomes in female plant are XXXXXX, whereas in case of male it may be either XXXXXY or XXXXY (McNeilage 1997; Gill et al. 1998; Shirkot et al. 2002). The fruit is refreshing and has high nutritive as well as medicinal value. The kiwifruit is the rich source of antioxidants and an excellent source of potassium, folate and vitamin C and E (Ferguson 1990; Ferguson and MacRae 1992; Wang et al. 1996; Shirkot et al. 2002).

Kiwifruit is very popular in low and mid-Himalayan region and for successful breeding of this plant identification of male and female genotypes is very crucial before orchard plantation. In twentieth century, two different RAPD primers were selected showing differential banding pattern between male and female genotypes of kiwifruit

and subsequently SCAR markers were developed (Gill et al. 1998). SCAR marker generated from one of the RAPD (SmX) was converted to a dominant marker, whereas the SCAR marker generated from another one (SmY) failed to show any polymorphism between male and female plants. Later on, another group of researchers identified two male-specific and six female-specific RAPD markers in this fruit crop (Shirkot et al. 2002).

Screwspine (*Pandanus species*)

The screwspine or *Pandanus* species, usually called *kewda* in India, is a dioecious perennial species predominantly found in coastal regions. It belongs to the family Pandanaceae. The somatic chromosome complement is $2n = 60$ and the female plants with the 6.15 pg genome, are bigger than the male ones possessing 5.09 pg genome (Panda et al. 2010). It is readily propagated from seeds but farmers generally do stem cuttings. The plant can grow in wild withstanding drought, strong wind and salt and it is traditionally used by the farmers of India. The leaves are used in making baskets, mats, hats, and roof thatch. The leaves of some species are as fragrant as basmati rice and hence it is used as a flavouring agent in the cuisines. The aromatic essential oil (*rhu*) is found from the inflorescence of the male plant. It has several medicinal properties such as the fresh leaves are used to treat sore throat and oil prepared from the young leaves is applied to cure burns while the bark is used in curing diarrhoea, dysentery and enteritis. The powder of the roots is used in treating leucorrhoea, abscess and oedema and to prevent miscarriage among the tribal community. Fibres extracted from the aerial roots are used to make cords and brushes and some species are used as starchy food sources. Chong et al. (2012) induced apoptosis on non-hormone dependent breast cancer cell with the application of ethanol extract of *P. amaryllifolius* leaves, hence there is anticancerous property in *Pandanus* leaves.

Very negligible work has been carried out in this crop regarding the sex determination at molecular level. For early determination of sex, Vinod et al. (2007) converted a 1263 bp amplicon generated from a male-specific RAPD primer (OPD-08) into an SCAR marker (MSSR-01) of 976 bp size in *P. fascicularis* L. Another group reported two male-specific RAPD markers viz., OPA-12 and OPN-18 producing 1150 and 600 bp fragment, respectively, in *P. tectorius* Parkinson (Panda et al. 2010).

Jojoba [*Simmondsia chinensis* (Link) Schneider]

Jojoba [*Simmondsia chinensis* (Link) Schneider] belongs to the family Simmondsiaceae and it is a dioecious shrub especially acclimatized for the desert and it is an emerging

cash crop in India, producing commercially valuable seeds. In 1978 Jojoba was introduced from Israel into India. The properties of the liquid wax stored in the seeds resemble to the spermaceti of the sperm whale which can be an alternative to the petro-products as well as lubricants for heavy machinery and are used in cosmetic, pharmaceutical and plastic industries. The seed wax of jojoba has some outstanding physical properties with highest limit viz. viscosity index, flash and fire points, dielectric constant, stability and freezing point. The wax can withstand repeated heating to temperatures above 285 °C. As jojoba oil is non-toxic, biodegradable and quite stable, it is used in making detergent, leather, plastic, pharmaceutical and cosmetic products worldwide. In desert areas, the jojoba plant provides shade from its canopy and eliminates windy erosion. The seed is the main propagule for jojoba and the plant takes 3–4 years to start flowering. Jojoba is a slow developing plant showing a male dominated (male:female::5:1) population (Agrawal et al. 2007). Due to this male biased population and the ability of one male to pollinate many females in jojoba, the Indian farmers have to eliminate a huge number of male plants for maximizing the number as well as growth of commercially important female plants.

Determination of sex in jojoba at the juvenile stage has immense importance in terms of commercial aspects. Although sexual differentiation of jojoba seedlings cannot be done cytologically since jojoba sex chromosomes are not distinguishable through cytological methods (Hosseini et al. 2011), a number of molecular markers have been employed for determination of sex in jojoba. Molecular study by Agrawal et al. (2007) identified a male-specific RAPD marker (OPG-05) producing 1400 bp amplicon in *S. chinensis* (Link) Schneider. Another study documented two male-specific and one female-specific RAPD markers along with PCR-based sex determination in jojoba by sex-determining region Y (Sry) gene primers and random primers (Mohasseb et al. 2009). Hosseini et al. (2011) identified one female-specific marker (F1) producing 460 bp fragment and one male-specific RAPD marker (F10) producing 680 bp fragment in *S. chinensis* cv. Arizona. Further researches developed male-specific 525 and 325 bp AFLP markers utilizing *EcoRI*-GC/*MseI*-GCG and *EcoRI*-TAC/*MseI*-GCG primer sets, respectively, and a female-specific 270 bp AFLP marker utilizing *EcoRI*-TAC/*MseI*-GCG primer set (Agarwal et al. 2011). Sharma et al. (2008) produced a male-specific ISSR having sequence (AG)₈T producing 1200 bp fragment in *S. chinensis* (Link) Schneider, whereas the same ISSR primer amplified of around 1.1 kb in male jojoba plants by Jangra et al. (2014) and subsequently converted it into an SCAR marker (GenBank accession number HQ166029.1) producing 1000 bp fragment. Heikrujam et al. (2014a, 2014b) successfully

converted two male-specific ISSR markers viz. (CA)₈G producing 1500 bp fragment and (CAC)₃GC producing 1300 bp fragment into male locus-specific STS markers, STS 807 of size 800 bp (JMSM) and STS VIS11 of size 584 bp in *S. chinensis* (Link) Schneider.

Yam (*Dioscorea* species)

Yam (*Dioscorea* species) is well known as *ratalu* in India and it belongs to the Dioscoreaceae family. It is a deciduous perennial herbaceous vine and is considered as a crop for subsistence agriculture. Yam is propagated vegetatively from tuberous roots and also through vine cutting while some species produce bulbils in leaf axils also. The axillary spikes bear the female flowers whereas the male flowers arise on long axillary panicle. Bhowmick et al. (1993) reported that as the basic chromosome number (x) is 9 or 10, the ploidy level varies from diploid onwards. Another group documented $x = 10$ and they also mentioned the presence of one or two extra chromosomes (B chromosomes) as large as the nuclear chromosomes in yam (Dansi et al. 2000). According to Bhat and Bindroo (1980), the male and female plants of *D. deltoidea* Wall. have homomorphic (ZZ) and heteromorphic sex chromosomes (ZW), respectively. In contradiction to that, another group suggested that the male yam plant might be of heterogametic sex (XY) while the female plants would be of homogametic sex (XX) in nature (Terauchi and Kahl 1999).

The edible yams are *D. alata* L., *D. esculenta* (Lour.) Burk. and *D. rotundata* (L.) Poir. Starchy tuber of yam is consumed after roasting and boiling and it can also be used to produce chips, flakes and flour. There are many poisonous yams that are used as insecticide or as an aid in hunting, fishing as well as arrow poisoning and clarified tubers can be consumed as famine food also (Bhowmick et al. 1993). Dutta (2015) reported several medicinal properties of yam. Yam tuber can be used as coolant for lowering body temperature and alcohol can be prepared from yam tuber as well. Most species of yam have steroid saponin like diosgenin and glucoside saponin from which many steroidal hormones are manufactured and used as anti-inflammatory, androgenic, estrogenic and contraceptive drugs. Yam bulbils are used to cure typhoid in children while the tubers maintain kidney functions, reduce blood cholesterol, regulate female sex hormones, reduce body weight and used against chronic diarrhoea, diabetes, tuberculosis, digestive disorders as well as respiratory disorders. Additionally, it can be used to prevent early miscarriage and it is useful for labour pain also. It is used for the treatment of insect, snake or scorpion bites. The paste prepared from yam tuber can be a good remedy for

various skin diseases, leprosy, gonorrhoea and wounds due to cancer. For quick recovery of fractured bones of animals, tuber decoction is applied.

Very limited work has been conducted for identifying the sex-specific marker in yam. An approach was taken for mapping *D. tokoro* genome using 271 AFLPs and 5 STSs, along with one biochemical and one phenotypic marker. The study identified 10 AFLP markers showing heterozygosity only in the male parent that depicted tight linkages of those molecular markers with the sex to their offspring (Terauchi and Kahl 1999).

Hemp (*Cannabis sativa* L.)

Hemp (*C. sativa* L.) is a dioecious species from the family Cannabaceae. It is one of the ancient plants adopted for its fibre and medicinal values as well as for its utility as an addictive and psychoactive drug. Hemp is also valued in paper industries and for the oil present in the seeds. According to Mandolino et al. (1999), hemp has chromosome number $2n = 20$. In general, *C. sativa* males are preferred over females for fibre quality (Horkay and Bocsa 1996). The females are homomorphic (XX) while the males are heteromorphic (XY) in nature with subtelocentric and strongly heterochromatic Y chromosome rich in repetitive sequences (Moliterni et al. 2004). This Y chromosome typically has a satellite at the short arm end and the extra genome size in male (1683 Mbp) over the female (1636 Mbp) is due the long arm of the Y chromosome (Sakamoto et al. 1998). Moreover, the X chromosome, submetacentric in nature, bears a satellite at its short arm end. An X:autosome dosage controls sex determination in *C. sativa* (Westgaard 1958; Grant et al. 1994). Truta et al. (2002) found a richer spectrum of esterase as well as peroxidase isoenzyme in male plants than the females. *C. sativa* has flexibility towards bearing bisexual flowers, i.e. monoecious plants can be found along with dioecious ones. Monoecious cultivars of hemp are useful in seed production and stem harvesting. Although there are no specific reports regarding the chromosome of monoecious hemp (Moliterni et al. 2004), it has been reported that the monoecious hemp cultivars have the XX constitution with a genetic basis for sex expression (Faux et al. 2014).

Several studies were carried out for determining the sex in this controversial crop. Sakamoto et al. (1995) reported two RAPD primers producing 500 and 730 bp amplicons specific for male sex; and the 730 bp DNA fragment was named MADC1 (male-associated DNA sequence in *C. sativa*). FISH by Sakamoto et al. (2000), using MADC1 and its flanking DNA as probes, revealed that a specific LINE-like retrotransposon accumulated at the terminal region of the long arm of the Y chromosome. Another

group also reported a male-specific 400 bp RAPD marker (MADC2) and converted it into an SCAR marker producing 390 bp amplicon (Mandolino et al. 1999). Sakamoto et al. (2005) found two male sex-linked RAPD (MADC3 and MADC4) markers encoding gag/pol polyproteins of copia-like retrotransposon and generating a doublet signal at the long arm end of Y chromosomes when used as probe in FISH study. They also reported that the heteromorphism in the sex chromosome might be due to the accumulation of retrotransposable elements on the Y chromosome. Heretofore, two RAPD markers viz. MADC5 and MADC6 (producing 961 and 151 bp fragments, respectively) specific to the male plants were converted into SCAR markers by Törjék et al. (2002) and those SCAR markers were able to generate 323 and 119 bp amplicon, respectively.

Conclusion and outlook

The system of sex identification in many underutilized dioecious plants remains unexplained till date; probably owing to the fact that majority of the domesticated plants are monoecious. The wild relatives of black pepper (*P. nigrum* L.) and wild grape (*Vitis vinifera* subsp. *Sylvestris*) are dioecious but their cultivated forms are bisexual and famous worldwide. In addition to that, most of the cultivated types of black pepper are protogynous with female phase preceding the male phase by few days to few weeks (Ravindran et al. 2000). The spinach, (*Spinacia oleracea*), belonging to the family Amaranthaceae, is another popular dioecious vegetable; but a monoecious plant, from the same family, *Beta vulgaris* or palak or spinach beet is dominant in Indian market substituting the spinach. The strawberry species are generally dioecious but the common cultivated strawberry (*Fragaria* × *ananassa*) belonging to Rosaceae (Gantait et al. 2010) is a hybrid plant and is monoecious in nature. The papaya is generally a monoecious species but it behaves as trioecious. In India, there is a trend to impose stress to the rarely found non-bearing papaya plant, which is supposed to be male, either by de-heading or inserting wooden stick into the stem; the plant starts fruiting later on i.e., the plant converts itself into the most common monoecious form. Obviously, there is an indication of sex reversal and it should be an important area of research in near future. Hence, there is a wide extent to be explored concerning the actual mechanism of dioecy, monoecy or trioecy in plants and more reliable and robust molecular markers are needed to sexually discriminate crops at an early stage. This review will be of significant to execute different strategies taken with respect to the sex determination of commercially important dioecious crops

in India and it will provide recent updates regarding the importance as well as sex-oriented studies in those crops.

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Compliance with ethical standards

Conflict of interest We, the authors of this article, declare that there is no conflict of interest and we do not have any financial gain from it.

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