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

Sutanu Sarkar¹ ² · Joydeep Banerjee¹ ³ · Saikat Gantait¹ ⁴

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...
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 Khryatin 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 elatum (squirtling 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.), teasel gourd, ivy gourd (Coccinia indica Wight and Arn. and C. grandis (L.) Voigt), kiwifruit (Actinidia delicosa var. delicosa) 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 triecious 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 Yβ were reported. Y chromosome determines the development of male flowers whereas Yβ 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 hastatus 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 kahrf 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 cardiotonic, antileucers and anthelmentic 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
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

![Diagram of plant sex chromosome evolution](image)

**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.
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, antidysslipidemic, antiinflammatory, analgetic, 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*.

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

| Dioecious plants | Major growing states | Major economic importance in India |
|------------------|----------------------|----------------------------------|
| *Trichosanthes dioica* | West Bengal, Assam, Tripura, Arunachal Pradesh, Orissa, Jharkhand and Bihar | Used as vegetable |
| *Coccinia* species | West Bengal, Tripura and Assam | Used as vegetable |
| *Momordica* 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 |
| *Pandanus* species | Orissa and West Bengal | Used as condiment |
| Jojoba | Rajasthan | Used in oil and cosmetic industry |
| Yam | Orissa | Used as starchy food |
| Hemp* | – | – |

* Cultivation of hemp in India is prohibited by law
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 CgMSM) 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. salhyadrca*, and *M. cochinchnensis* (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. cochinchnensis*) 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 tease gourd and fruit; young twigs as well as leaves are widely consumed as vegetables.

The *M. dioica* has several medicinal uses (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 males, 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.

Asparagus officinalis (Asparagus officinalis L.)

Garden asparagus (*Asparagus 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 (Reamun-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 homoyzogous 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 (Li 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 plagiotropic (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, gums 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 OB-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 OPG02 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 (GA6CC) and UBC-852 (TC6RA), respectively and a female-specific amplification of 636 bp fragment using ISSR-23 (GACACA6CC) 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
**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 (Farooqui 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 (Farooqui 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 (OP008) 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)$_6$CG and (AC)$_6$TA, 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 (Esfandiyari 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 Arecales/Phoenix/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 anti-infective, anti-inflammatory and anti-hemorrhagic, 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 world-wide 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 delicosa var. delicosa)**

Chinese gooseberry or kiwifruit (*Actinidia delicosa* var. *delicosa*) 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. delicosa* var. *delicosa* and *A. chinensis* are the only two species bearing edible fruits. Furthermore, *A. delicosa* (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. delicosa* var. *delicosa* (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 XXXXXX or XXXXYY (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).

**Screwpine (Pandanus species)**

The screwpine 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 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)$_9$T 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)$_3$G producing 1500 bp fragment and (CAC)$_2$GC 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. deltoides* 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 sapogenins like diosgenin and glycoside saponin from which many steroidal hormones are manufactured and used as antiinflammatory, 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 Bocska 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. sativus* (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. sativus* 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 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 x 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 tricocious. 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 deheading 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 tricoccy 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.

**Acknowledgements** The authors would like to acknowledge Directorate of Research and Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India for providing library assistance for preparation of this review article.

**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.

**References**

Adhikari S, Saha S, Bandyopadhyay TK, Ghosh P (2014) Identification and validation of a new male sex-specific ISSR marker in pointed gourd (Trichosanthes dioica Roxb.). Sci World J (Article ID 216886), doi:10.1155/2014/216886

Agarwal M, Shirivastava N, Padh H (2011) Development of sex-linked AFLP markers in Simmondsia chinensis. Plant Breed 130:114–116

Agrawal V, Sharma K, Gupta S, Kumar R, Prasad M (2007) Identification of sex in Simmondsia chinensis (jojoba) using RAPD markers. Plant Biotechnol Rep 1:207–210

Ahire YR, Deokule SS (2012) Screening of allelopathic activity of Momordica dioica and Mucia madraspatana. Res Rev 1:15–21

Ainsworth C (2000) Boys and girls come out to play: the molecular biology of dioecious plants. Ann Bot 86:211–221

Ainsworth C, Rahman A, Parker J, Edwards G (2005) Intersex inflorescences of Rumex acetosa demonstrate that sex determination is unique to each flower. New Phytol 165:711–720

Al-Mahmoud ME, Al-Dous EK, Al-Azwani EK, Malek JA (2012) DNA-based assays to distinguish date palm (Areccaceae) gender. Am J Bot 99:e7–e10

Aoki H, Kieu NT, Kuze N, Tomisaka K, Chuyen NV (2002) Carotenoid pigments in gac fruit (Momordica cochinchinensis Spreng.). Biosci Biotechnol Biochem 66:2479–2482

Banerjee NS, Prasad M, Das MR (1999) Male-sex-associated RAPD markers in Piper longum. Curr Sci 77:693–697

Banu SG, Kumar G, Rajasekara PM (2007) Cholesterol-lowering activity of the aqueous fruit extract of Trichosanthes dioica Roxb. (L.) in normal and streptozotocin diabetic rats. J Clin Diag Res 1:561–569

Baratkke RC, Patil CG (2009) Identification of a RAPD marker linked to sex determination in Momordica dioica Roxb. Ind J Genet 69:254–255

Barbour JR, Read RA, Barnes RL (2008) Moraceae-Mulberry family Morus L. Mulberry. In: Bonner FT, Karrfalt RP (eds) United States Department of Agriculture Forest Service Agriculture Handbook 727-The Woody Plant Seed Manual, pp 728–732

Barrett SCH (2002) The evolution of plant sexual diversity. Nat Rev Genet 3:274–284

Barrett SCH (2013) The evolution of plant reproductive systems: how often are transitions irreversible? Proc Roy Soc Bot 280:20130913. doi:10.1098/rspb.2013.0913

Bergero R, Qiu S, Forrest A, Borthwick H, Charlesworth D (2013) Expansion of the pseudoautosomal region and ongoing recombination suppression in the Silene latifoliax chromosomes. Genetics 194:673–686

Bharathi LK, Munshi AD, Vinod Chandrashekaran S, Behera TK, Das AB, John KJ, Vishalnath (2011) Cytotaxonomical analysis...
of *Momordica* L. (*Cucurbitaceae*) species of Indian occurrence. J Genet 90:21–30

Bhat BK, Bindroo BB (1980) Sex Chromosomes in *Dioscorea deltoidea* Wall. Cytologia 45:739–742

Bhattacharya E, Ranade SA (2001) Molecular distinction amongst varieties of mulberry using RAPD and DAMD profiles. BMC Plant Biol 1:3–10

Bhowmick BK, Nanda S, Nayak S, Jha S, Joshi RK, Bose TK, Maharana T, Sen H (1993) Yam. In: Bose TK, Som MG, Kabir J (eds) Vegetable crops. Naya Prakash, Kolkata, pp 879–888

Bhowmick BK, Nanda S, Nayak S, Jha S, Joshi RK (2014) An APETALA3 MADS-box linked SCAR marker associated with male specific sex expression in *Coccinia grandis* (L.) Voigt. Sci Hortic 176:85–90

Charlesworth B (1991) The evolution of sex chromosomes. Science 251:1030–1032

Chattopadhyay PK, Bose TK (2006) Palmyra palm. In: Parthasarathy VA, Chattopadhyay PK, Bose TK (eds) Plantation crops (Volume 2). Naya Udyog, Kolkata, pp 493–511

Cherif E, Zehdi S, Castillo K, Chabriallange N, Abdouilkader S, Pintaud JC et al (2013) Male-specific DNA markers provide genetic evidence of an XY chromosome system a recombination arrest and allow the tracing of paternal lineages in date palm. New Phytol 197:409–415

Chirathaworn C, Kongcharoensuntorn W, Dechdoungchan T, Lowan-Thamcharoen T, Lotowitchapat A, Sa-Ngunanoo P, Povorovaray Y (2007) *Myristica fragrans* Houtt. methanolic extract induces apoptosis in a human leukemia cell line through SRT1 mRNA downregulation. J Med Assoc Thai 90:2422–2428

Chong HZ, Yap SK, Rahmat A, Alitheen NB, Othman F, Akim AM, Grant S, Houben A, Siroky J, Pan WH, Macas J, Saedler H, Vyskot B, Horkay E, Bocsa I (1996) Objective basis for evaluation of different mechanisms of sex determination and sex-linked PCR markers for gender identification in *Actinidia*. Theor Appl Genet 97:439–445

Grant S, Houben A, Vyskot B, Siroky J, Pan WH, Macas J, Saedler H (1994) Genetics of sex determination in flowering plants. Dev Gen 15:214–230

Harandi OF, Ghaffari M (2001) Chromosome studies on pistachio (*Pistacia vera* L.) from Iran. In: Ak BE (ed) XI GREMPA seminar on pistachios and almonds. CIEHAE, Zaragoza, pp 35–40

Harkess A, Mercati F, Shan HY, Sunseri F, Falavigna A, Leebens-Mack J (2015) Sex-biased gene expression in dioecious garden asparagus (*Asparagus officinalis*). New Phytol 207:883–892

Harvey CF, McNeile MA, Gill GP, Fraser LG (1997) Sex determination in *Actinidia* 1. Sex-linked markers and progeny sex ratio in diploid *A. chinensis*. sex. Plant Reprod 10:149–154

Hosseini FS, Hassani HS, Arvin MJ, Baghizadeh A, Mohammadi-Nejad G (2011) Sex determination of jojoba (*Simmondsia chinensis*) using bulked segregant analysis. Theor Appl Genet 89:9–13

Hormaza JJ, Dollo L, Polito VS (1994) Identification of RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. Theor Appl Genet 89:9–13

Hossinei FS, Hassani HS, Arvin MJ, Baghizadeh A, Mohammadi-Nejad G (2011) Sex determination of jojoba (*Simmondsia chinensis* cv Arizona) by random amplified polymorphic DNA (RAPD) molecular markers. Afr J Biotech 10:470–474
Hough I, Ägren IA, Barrett SCH, Wright SI (2014) Chromosomal distribution of cytonuclear genes in a dioecious plant with sex chromosomes. Genome Biol Evol 6:2439–2443

Hussain A, Wahab S, Rizvi A, Hussain MS (2011) Macroscopic, anatomical and physico-chemical studies on leaves of Coccinia indica Wight and Arn., growing wildly in eastern Uttar Pradesh region of India. Ind J Nat Prod Resour 2:74–80

Ii Y, Ugarami A, Uno Y, Kanechi M, Inagaki N (2012) RAPD based analysis of differences between male and female genotypes of Asparagus officinalis. Hortic Sci (Prague) 39:33–37

Indira EP, Anto PV (2002) Karyotype analysis in Calamus palustris Griff. J Bamboo Rattan 1:199–203

Jamsari A, Nitz I, Reamon-Buttner SM, Jung C (2004) BAC derived diagnostic markers for sex determination in asparagus. Theor Appl Genet 108:1140–1146

Jangra S, Kharb P, Mitra C, Uppal S (2014) Early diagnosis of sex in jojoba Simmondsia chinesis (Link) Schneider by sequence characterized amplified region marker. Proc Nat Acad Sci 84:251–255

Jiang C, Sink KC (1997) RAPD and SCAR markers linked to the sex expression locus M in Asparagus. Euphytica 94:329–333

Kabir J (1993) Asparagus. In: Bose TK, Som MG, Kabir J (eds) Vegetable crops. Naya Udyog, Kolkata, pp 889–899

Kafkas M, Khodaeimaminjan M, GÜney M, Kafkas E (2015) Identification of sex-linked SNP markers using RAD sequencing suggests ZW/ZZ sex determination in Pustacia vera L. BMC Genom 16:98–108

Kanno A, Kubota S, Ishino K (2014) Conversion of a male-specific RAPD marker into an STS marker in Asparagus officinalis L. Euphytica 197:39–46

Karmakar K, Sinha RK, Sinha S (2013) Karyological and electrophoretic distinction between sexes of Trichosanthes bracteata. Am J Plant Sci 4:494–497

Khadke GN, Bindu KH, Ravishankar KV (2012) Development of SCAR marker for sex determination in dioecious betel vine (Piper betle L.). Curr Sci 103:712–716

Kumar S, Singh BD, Pandey S, Ram D (2008) Inheritance of leaf and stem morphological traits in pointed gourd (Trichosanthes dioica Roxb.). J Crop Improv 22:225–233

Kumar S, Singh BD, Sinha DP (2012) RAPD markers for identification of sex in pointed gourd (Trichosanthes dioica Roxb.). Ind J Biotech 11:251–256

Kumar S, Kumar R, Sinha V (2014) Genetics of dioecy and causal sex chromosomes in plants. J Genet 93:241–277

Latha PG, Sindhu PG, Suja SR, Geetha BS, Pushpangadan P, Rajasekharan S (2005) Pharmacology and chemistry of Myristica fragrans Houtt.—a review. J Spices Arom Crop 14:94–101

Law TF, Lebel-Hardenack S, Grant SR (2002) Silver enhances stamen development in female white campion (Silene latifolia [Caryophyllaceae]). Am J Bot 89:1014–1020

Li M, Yang H, Li F, Yang F, Yin G, Gan S (2010) A malespecific SCAR marker in Calamus simplicifolius, a dioecious rattan species endemic to China. Mol Breed 25:549–551

Maiti S (2006) Betel vine. In: Parthasarathy VA, Chattopadhyay PK, Bose TK (eds) Plantation crops, vol 2. Naya Udyog, Kolkata, pp 463–491

Manoj P, Banerjee NS, Ravichandran P (2005) Development of sex-associated SCAR markers in Piper longum L. PGR NewsL 14:44–50

Mathew LS, Spannag M, Al-Malki A, George B, Torres MF, Al-Dous EK et al (2014) A first genetic map of date palm (Phoenix dactylifera) reveals long-range genome structure conservation in the palms. BMC Genom 15:285–294

McNeilage MA (1997) Progress in breeding hermaphrodite kiwifruit cultivars and understanding the genetics of sex determination. Acta Hortic 444:72–78

Milewicz M, Sawicki J (2012) Mechanisms of sex determination in plants. Cas Slez Muz Opava (A) 61:123–129

Ming R, Wang J, Moore PH, Paterson AH (2007) Sex chromosomes in flowering plants. Am J Bot 94:141–156

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annu Rev Plant Biol 62:485–514

Mishra D, Shukla AK, Tripathi KK, Singh A, Dixit AK, Singh K (2006) Efficacy of application of vegetable seed oils as grain protectant against infestation by Callosobruchus chinensis and its effect on milling fractions and apparent degree of dehulking of legume-pulses. J Oleo Sci 56:1–7

Mohan RHY, Tandon R (1997) Bamboo and rattans: from riches to rags. Proc Nat Acad Sci-India 63:245–267

Mohasseb HBA, Moursy HA, El-Bahr MK, Adam ZM, Solliman M (2009) Sex determination of jojoba using RAPD markers and Sry gene primer combined with RAPD primers. Res J Cell Mol Biol 3:102–112

Moliterni CMV, Cattivelli L, Ranalli P, Mandolino G (2004) The sexual differentiation of Cannabis sativa L., a morphological and molecular study. Euphytica 140:95–106

Nakayama H, Ito T, Hayashi Y, Sonoda T, Fukuda T, Ochiai T, Kameya T, Kanno A (2006) Development of sex-linked primers in garden asparagus (Asparagus officinalis L.). Breed Sci 56:327–330

Nanda S, Kara B, Nayaka S, Jha S, Joshi RK (2013) Development of an ISSR based STS marker for sex identification in pointed gourd (Trichosanthes dioica Roxb.). Sea Hortic 150:11–15

Narasimhan S, Kannan S, Ilango K, Maharajan G (2005) Antifeedant activity of Momordica dioica fruit pulp extracts on Spodoptera litura. Fitoterapia 76:715–717

Nath V, Kumar D, Pandey V (2008) Fruits for the future, Vol 1: well versed arid and semi arid fruits. Satish Serial Publishing House, Delhi

Ottman Y (1987) Rediscovering the realm of fruiting mulberry varieties. Fruit Var J 41:4–7

Panda KK, Sahoo B, Das AB, Pandaa BB (2010) Use of RAPD markers to detect sex differences in Pandanus tectorius Parkinson, an important bioresource plant in Orissa, India. Int Biodivers Sci Ecosyst Serv Manag 6:28–34

Patil CG, Baratarkale RC, Sandljwad AM (2012) Development of a RAPD-based SCAR marker for sex identification in Momordica dioica Roxb. Israel J Plant Sci 60:457–465

Pekanwar SS, Kalyankar TM, Kokate SS (2013) Pharmacological activities of Coccinia grandis: review. J Appl Pharm Sci 3:114–119

Prajapati ND, Purohit SS, Sharmi AK, Kumar TA (2003) Handbook of medicinal plants: a complete source book. Agrobios (India) Shyam Printing Press, Jodhpur

Rai PK, Jaiswal D, Singh R, Watal G (2008) Glycemic property of Trichosanthes dioica leaves. Pharm Biol 46:894–899

Rai PK, Shukla S, Mehta S, Rai NK, Rai AK, Watal G (2010) Therapeutic phytoelemental profile of Trichosanthes dioica. Adv Mater Lett 1:210–216

Rai MP, Thilakchand KR, Palatty PL, Rao P, Rao S, Bhat HP et al (2011) Piper betel Linn. (betel vine), the maltigned southeast asian medicinal plant possesses cancer preventive effects: time to reconsider the wronged opinion. Asian Pacific J Cancer Prev 12:2149–2156

Rathore DS (1991) Kiwifruits. In: Mitra SK, Rathore DS, Bose TK (eds) Vegetable crops. Naya Prokash, Kolkata, pp 893–899

Ravindran PN, Nirimal BK, Sasikumar B, Krishnamoorthy KS (2000) Botany and crop improvement of black pepper. In: Ravindran...
Sakamoto K, Abe T, Matsuyama T, Yoshida S, Ohmido N, Fukui K, Sami SA, Jiming J, Mohamed AM (2014) Identification of novel sex-specific DNA markers to distinguish the genders in dioecious plants, Cannabis sativa L. Plant Mol Biol 4:91–98

Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. Am J Bot 82:596–606

Roser M (1994) Pathways of karyological differentiation in palms. Plant Syst Evol 189:83–122

Sakamoto KL, Shimomura K, Kamada H, Satoh S (2000) Site-specific accumulation of a LINE-like retrotransposon in a sex chromosome of the dioecious plant Cannabis sativa. Plant Mol Biol 44:723–732

Sakamoto K, Abe T, Matsuyama Y, Yoshida S, Ohmido N, Fukui K, Satoh S (2005) RAPD markers encoding retrotransposable elements are linked to the male sex in Cannabis sativa L. Genome 48:931–936

Samantaray S, Phuralalpatnam A, Bishoyi AK, Geetha KA, Maiti S (2012) Identification of sex-specific DNA markers in betel vine (Piper betle L.), Genet Resour Crop Evol 59:645–653

Sami SA, Jiming J, Mohamed AM (2014) Identification of novel sex-specific PCR-based markers to distinguish the genders in Egyptian date palm trees. Asia Int J Agri Sci Res 4:45–54

Sarkar AK, Datta N (1985) Cytology of Cannabis sativa L. (Cannabis L., palmaceae) as an aid to their taxonomy. Cell Chrom Res 8:69–73

Sarmah P, Sarma RN (2011) Identification of a DNA marker linked to sex determination in Calamus tenuis Roxb., an economically important rattan species in northeast India. Mol Breeder 27:115–118

Seefelder S, Ehrmaier H, Schweizer G, Seigner E (2000) Male and female genetic linkage map of hops, Humulus lupulus. Plant Breeder 119:249–255

Sharma DR, Shirkot P (2004) Biotechnological interventions for genetic amelioration of Actinidia delicosa var. delicosa (kiwifruit) plant. Ind J Biotech 3:249–257

Sharma G, Pandey DN, Fant MC (1990) The biochemical evaluation of feeding Trichosanthes dioica Roxb. seeds in normal and mild diabetic human subjects in relation to lipid profile. Ind J Physiol Pharmacol 34:140–148

Sharma K, Agrawal V, Gupta S, Kumar R, Prasad M (2008) ISSR marker-assisted selection of male and female plants in a dioecious crop jojoba (Simmondsia chinensis). Plant Biotechnol Rep 2:239–243

Sheeba TE, Bindu KH, Anto P, Dhanya K, Siju S, Kumar TV (2013) A SCAR marker based method for sex determination in dioecious betel vine (Piper betle). Ind J Agri Sci 83:1409–1410

Shephard H, Parker J, Darby P, Ainsworth CC (1999) Sex expression in hop (Humulus lupulus L. and H. japonicas Sieb. et. Zucc.): floral morphology and sex chromosomes. In: Ainsworth CC (ed) Sex determination in plants. BIOS Scientific Publishers, Oxford, pp 137–148

Shephard HL, Parker JS, Darby P, Ainsworth CC (2000) Sexual development and sex chromosomes in hop. New Phytol 148:397–411

Shibu MP, Ravishankar KV, Anand L, Ganeshraiah KN, Shaananker U (2000) Identification of sex-specific DNA markers in the dioecious tree, nutmeg (Myristica fragrans Houtt.). PGR Newslett 121:59–51

Shirkot P, Sharma DR, Mohapatra T (2002) Molecular identification of sex in Actinidia delicosa var. delicosa by RAPD markers. Sci Hortic 94:33–39

Shivhare Y, Singour PK, Patil UK, Pawar RS (2010) Wound healing potential of methanolic extract of Trichosanthes dioica Roxb. (fruits) in rats. J Ethnopharmacol 127:614–619

Singh M, Kumar S, Singh AK, Ram D, Kalloo G (2002) Female sex-associated RAPD marker in pointed gourd (Trichosanthes dioica Roxb.). Curr Sci 82:131–132

Singh P, Kharb P, Pandey N, Batra P, Khatak S, Dhillon S et al (2006) RAPD analysis for genetic diversity and identification of sex-specific marker in date palm. Haryana J Hortic Sci 35:232–234

Sinha S, Guha A, Sinha B, Sinha RK, Banerjee N (2007) Average packing ratio and evolution of sex chromosomes in dioecious Coccinia indica and Trichosanthes dioica. Cytologia 72:369–372

Soldatova NA, Khryanin VN (2010) The effects of heavy metal salts on the phytomorphological status and sex expression in Marijuana. Russ J Plant Physiol 57:96–100

Souza A, Fuchs J, Renner SS (2013) Molecular cytogenetics (FISH, GISH) of Coccinia grandis: a ca. 3 myr-old species of cucurbitaceae with the largest Y/autosome divergence in flowering plants. Cytogenet Genome Res 139:107–118

Talukdar SN, Hossain MN (2014) Phytochemical, phytotherapeutical and pharmacological study of Momordica charantia: a review. Asian Pacific J Biomed 4:5299–5302

Telgmann-Rauber A, Jamsari A, Kinney MS, Pires JC, Jung C (2007) Genetic and physical maps around the sex-determining M-locus of the dioecious plant asparagus. Mol Genet Genom 278:221–234

Terauchi R, Kahl G (1999) Mapping of the Dioscorea tokoro genome: AFLP markers linked to sex. Genome 42:752–762

Thakur BS, Rathore DS (1991) Pistachios. In: Mitra SK, Rathore DS, Phatak AS, Narayana R (eds) Advances in Horticulture. Malhotra Publishing House, New Delhi, pp 373–383

Thiruchitrambalam M, Shannugam D (2012) Influence of pre-treatments on the mechanical properties of palmyra palm leaf stalk fiber-polyester composites. J Reinf Plast Compos 31:1400–1414

Tilka EA, Namli S, Işıklan Ç (2011) Determination and assessment of the sex chromosomes of male trees of pistachio (Pistacia vera L.) using in vitro culture. Aust J Crop Sci 5:291–295

Török O, Buchern N, Kiss E, Homoki H, Finta-Korpolvaz L, Bocska I et al (2002) Novel male specific markers (MADC5, MADC6) in hemp. Euphytica 123:209–218

Truta E, Gille E, Toth E, Maniu M (2002) Biochemical differences in Cannabis sativa L. depending on sexual phenotype. J Appl Genet 43:451–462

Usha R, Indira VS, Jhansi S, Swamy PM (2009) Physiological and molecular variation among the two genders of Piper betle L. Nat Acad Sci Lett 32:93–98

Vijayan K, Nair CV, Chatterjee SN, Caspian J (2009) Diversification of mulberry (Morus indica var. s36), a vegetatively propagated tree species. Env Sci 7:23–30

Vinod MS, Raghavan PS, George S, Parida A (2007) Identification of a sex-specific SCAR marker in dioecious Pandanus fascicularis L. (Pandanaeaceae). Genome 50:834–839

Viswanathan TV (1995) Medicinal and aromatic plants. In: Chadha KL, Gupta R (eds) Advances in Horticulture. Malhotra Publishing House, New Delhi, pp 373–383

Vuong LT, Franke A, Custer L, Murphy SP (2006) Momordica dioica var. Roxb., an economically important plant species. Env Sci 7:23–30

Vinod MS, Raghavan PS, George S, Parida A (2007) Identification of a sex-specific SCAR marker in dioecious Pandanus fascicularis L. (Pandanaeaceae). Genome 50:834–839

Viswanathan TV (1995) Medicinal and aromatic plants. In: Chadha KL, Gupta R (eds) Advances in Horticulture. Malhotra Publishing House, New Delhi, pp 373–383

Vuong LT, Franke A, Custer L, Murphy SP (2006) Momordica cochinchinensis Spreng. (gac) fruit carotenoids reevaluated. J Food Compos Anal 19:664–668

Vysokt B, Hobza R (2004) Gender in plants: sex chromosomes are emerging from the fog. Trends Genet 20:432–438
Wang H, Cao GH, Prior RL (1996) Total antioxidant capacity of fruits. J Agri Food Chem 44:701–705
Wang J, Na J, Yu Q, Gschwend AR, Han J, Zeng F et al (2012) Sequencing papaya X and Y chromosomes reveals molecular basis of incipient sex chromosome evolution. Proc Nat Acad Sci 109:13710–13715
Westgaard M (1958) The mechanism of sex determination in dioecious plants. Adv Genet 9:217–281
Xu HC, Yin GT, Li YD, Fu JG, Zhang WL (2000) Distribution and utilization of rattans in China. In: Xu HC, Rao AN, Zeng BS, Yin GT (eds) Research on rattans in China, conservation, cultivation, distribution, ecology, growth, phenology, silviculture, systematic anatomy and tissue culture. IPGRI-APO, Serdang
Yang H, Gan S, Yin G, Hu H (2005) Identification of random amplified polymorphic DNA markers linked to sex determination in Calamus simplicifolius C.F.Wei. J Integr Plant Biol 47:1249–1253
Yang S, Kyun NM, Jang JP, Kyung AK, Kim BY, Sung NJ, Oh WK, Ahn JS (2006) Inhibition of protein tyrosine phosphatase 1B by lignans from Myristica fragrans. Phytother Res 20:680–682
Younis RAA, Ismail OM, Soliman SS (2008) Identification of sex-specific DNA markers for date palm (Phoenix dactylifera L.) using RAPD and ISSR techniques. R J Agri Biol Sci 4:278–284
Zheng L, Zhang YM, Zhan YZ, Liu CX (2014) Momordica cochinensis seed extracts suppress migration and invasion of human breast cancer ZR-75-30 cells via down-regulating MMP-2 and MMP-9. Asian Pacific J Cancer Prev 15:1105–1110