Plant Omics Journal POJ

POJ 6(2):144-149 (2013) ISSN:1836-3644

Review article

Sex-linked markers in dioecious plants

Monika Milewicz, Jakub Sawicki

Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn, Plac Łódzki 1, 10-727 Olsztyn, Poland

***Correspondent author: monika.milewicz@uwm.edu.pl; jakub.sawicki@uwm.edu.pl**

Abstract

Dioecy is generally associated with sexual dimorphism, which can be noticed with the naked eye, but in plants the sex of an individual, at the early stages of development before flowering, is difficult to diagnose. The situation is more complicated in bryophytes, where determination of sex in adult individuals can be problematic or even impossible. Here, molecular tools are helpful. This paper focuses on sex-linked molecular markers in plants. It presents plant species, for which sex-linked markers have been developed, indicates the most popular marker systems used in the research and tries to emphasise that some kind of information about sex determination can be provided during development of sex-linked markers.

Keywords: dioecious plants; female; male; marker systems; sex determination; sex-linked markers.

Introduction

Whereas only around 6% of angiosperms are dioecious (Renner and Ricklefs, 1995), over 50% of bryophytes develop sexual reproduction organs (antheridia and archegonia) on separate individuals (Shaw and Goffinet, 2000). Dioecy is generally associated with sexual dimorphism. This assumption holds true for most animal species, but in plants the sex of an individual is difficult to determine at the early stages of development before flowering. The situation is even more complex in bryophytes, where determination of sex in adult individuals can be highly problematic. Apart from a seasonal occurrence of sex organs (antheridia and archegonia) in this group of plants, sexual dimorphism is weakly expressed or non-existent. Beside this, sexual reproduction organs in bryophytes are sporadically developed, even in the season. Biochemical and cytological analyses do not always support sex determination in plants. Heteromorphic sex chromosomes are less popular in plants than in the animal kingdom. Morphologically different chromosomes have, however, been found in some plant species, including members of the genera: *Cannabis*, *Humulus*, *Rumex*, *Silene* (Parker, 1990) and three liverwort species: *Sphaerocarpos donnellii*, *Sphaerocarpos texanus* (Allen, 1917), *Marchantia polymorpha* (Bischler, 1986). An occurrence of heteromorphic sex chromosomes is also suspected in mosses of the genus *Macromitrium* (Ramsay, 1966). Early sex identification in plants is especially important for breeders. They attach a different value to male and female plants. Owing to their ability to produce seeds and fruit, female individuals are generally considered to be more valuable in agriculture. Species whose female representatives are more desirable in the production process include *Actinidia deliciosa* (Shirkot et al., 2002), *Borassus flabellifer* (George et al., 2007), *Carica papaya* (Parasnis et al., 2000), *Eucommia ulmoides* (Xu et al., 2004), *Hippophae rhamnoides* (Persson and Nybom 1998; Sharma et al., 2010), *Myristica fragrans* (Shibu et al., 2000), *Piper longum* (Manoj et al., 2005- 2008), *Pistacia vera* (Hormaza et al., 1994), *Phoenix dactylifera* (Younis et al., 2008) and *Simmondsia chinensis* (Agrawal et al., 2007; Sharma et al., 2008). In other species, male individuals are of a greater breeding value, among them *Asparagus officinalis* whose male individuals produce larger and thicker shoots (Gao et al., 2007). In non-crop plants, sex determination can be of environmental significance. It supports population studies which examine the proportions between male and female individuals and investigate factors that influence sex distribution. The results of such research are used to provide selected plants with the status of protected species. The methods of sex determination in plants are a popular topic of research among experts in various fields, including agriculture, horticulture, ecology and environmental protection.

Popular marker systems in search of difference between females and males

The discussed demand for tools supporting sex determination in plants gave rise to a series of molecular studies investigating DNA markers that could be used for that purpose. A molecular marker (DNA marker) is a DNA sequence observed in minimum two easy to distinguish versions (Brown, 2001), which reveals individual polymorphisms. The preferred marker should demonstrate the widest possible range of variation in the analyzed trait, and it should not be affected by environmental factors. An effective marker should guarantee reproducibility, and it should be easy to detect. Molecular markers facilitate analyses of variations between individuals, regardless of their development stage (Sztuba- Solińska, 2005), which is particularly useful in sex determination studies of plants. Some researchers have suggested that effective markers for plants should be relatively short to support sex determinations in herbarium specimens with damaged DNA. Shorter sequences increase the probability of successful amplification (Korpelainen et al., 2008).

Table 1. Sex determination methods and the number of identified sex-linked markers for various plant species.

Plant species	Marker system and other methods	Determined sex and number of discovered markers	References
Actinidia chinensis	RAPD	1F, 1M	Harvey et al. 1997
Actinidia deliciosa var. deliciosa	RAPD	6F, 2M	Shirkot et al. 2002
Asparagus officinalis	RAPD	2F, 2M	Jiang and Sink 1997; Gao et al. 2007
	RAPD-SCAR	1F, 1M	Jiang and Sink 1997; Gao et al. 2007
	RFLP	1F, 1M	Biffi et al. 1995
	AFLP	1M	Spada et al. 1998
Atriplex garrettii	RAPD-SCAR	1M	Ruas et al. 1998
Borassus flabellifer	RAPD	1M	George et al. 2007
Cannabis sativa	RAPD-SCAR	2M	Sakamoto 1995; Mandolino et al. 1999;
Carica papaya	RAPD-SCAR	1M	Parasnis et al. 2000
	ISSR	1 for both M and H; 1 for both F and H	Parasnis et al. 1999; Gangopadhyay et al. 2007
Commiphora wightii	RAPD	1F, 1H, 1 for both F and H	Samantaray et al. 2010
Cycas circinalis	RAPD	1F, 1M	Gangopadhyay et al. 2007
Dioscorea tokoro	AFLP	1M	Terauchi et al. 1999
Distichlis spicata	RAPD	1F	Eppley et al. 1998
Encephalartos natalensis	RAPD	1F	Prakash & Staden 2006
Eucommia ulmoides	RAPD-SCAR	1F	Xu et al. 2004
	AFLP-SCAR	1M	Wang et al. 2011
Ficus fulwa	AFLP	1M	Tracey et al. 2004
Ginkgo biloba	RAPD-SCAR	1F, 1M	Liao et al. 2009
	AFLP	3F, 1M	Wang et al. 2001
Hippophae rhamnoides	RAPD	2M	Persson and Nybon 1998; Sharma et al. 2010
	isozymes	1F	Sharma et al. 2010
Humulus lupulus	RAPD	3M	Seefelder et al. 2000
	ISSR	2M	Danilova and Karlov 2006
	AFLP	10M	Polley et al. 1997
Marchantia polymorpha	Representational Difference Analysis (RDA)	2F, 6M	Fujisawa et al. 2001
Melandrium album (Silene latifolia)	RAPD-SCAR	5M	Zhang et al. 1998
Melandrium rubrum (Silene dioica)	RAPD	1F, 1M	Di Stilio et al. 1998
Mercurialis annua	RAPD-SCAR	2M	Khadka et al. 2002
	Arbitrary primed-polymerase chain reaction (AP-PCR)	1F	Yang et al. 1998
Myristica fragrans	RAPD	1F	Shibu et al. 2000
Nyholmiella obtusifolia	ISSR	1F, 1M	Milewicz and Sawicki 2011
Piper longum	RAPD	2M	Banerjee et al. 1999
	RAPD-SCAR	1M	Manoj et al. 2005
	Differential Display	8F, 3M	Manoj et al. 2005-2008
Pistacia vera	RAPD	1F	Hormaza et al. 1994
Phoenix dactylifera	RAPD	3F, 2M	Younis et al. 2008
	ISSR	5M	Younis et al. 2008
Poa arachnifera Torr.	AFLP	2M	Renganayaki et al. 2005
Pseudocalliergon trifarium	ISSR-SCAR	1F	Korpelainen et al. 2008
Rumex acetosa	RFLP	1M	Ruiz et al. 1994
	FISH	1M	Shibata et al. 1999
	AFLP	4M	Rahman and Ainsworth 2004
Rumex nivalis	AFLP	1M	Stehlik and Blattner 2004
	AFLP-SCAR	1M	Stehlik and Blattner 2004
Salix viminalis	RAPD	1F	Alström-Rapaport et al. 1998
	RAPD-SCAR	2F	Gunter et al. 2003
Simmondsia chinensis (jojoba)	RAPD	1M	Agrawal et al. 2007
	ISSR	1M	Sharma et al. 2008
Sphaerocarpos texanus	RAPD	3F, 1M	McLetchie and Collins 2001
Trichosanthes dioica	RAPD	1F, 1M	Kumar et al. 2008
Uapaca kirkiana	AFLP	1F	Mwase et al. 2007

The most popular markers for sex determination in plants include RAPD (Random Amplified Polymorphic DNA), SCAR (Sequence-characterized Amplified Region), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism) and microsatellites (Table 1). The search for molecular markers has continued for many years (Table 1), and although RAPD was developed in the early 1990s (Williams et al., 1990), it continues to enjoy high popularity today. In the discussed studies, RAPD was the method of choice for determining the sex of investigated plant species. The above technique is simple, cheap and not very time consuming. It is a highly reliable method that enjoys greater popularity than the much older, and seemingly better studied technique of RFLP. RAPD requires much smaller DNA samples, and it is much cheaper than RFLP (Sztuba-Solińska, 2005). Obviously, the method has its disadvantages, which include poor reproducibility due to high sensitivity to changes in amplification conditions. For this reason, it is recommended that RAPD markers are converted to specific SCAR markers (Paran and Michelmore, 1993). Regardless of the applied marker systems, the search for genetic variations between the sexes should give rise to the marker mentioned above. Longer (20-30 bp) and more specific primers for these markers would facilitate amplification of the desirable sequence, and they would guarantee repeatability of measurement results. Furthermore, development of sex-linked SCAR markers support sex differentiation even of a single individual- the sex-specific band appears or does not. For other marker systems, which generate the whole band patterns, it would be difficult to identify a gender without the need of comparing the band patterns for both sexes. Unfortunately, new SCAR markers are rarely developed by scientists who investigate genetic differences between the sexes in plants (Table 1). Despite their usefulness, such markers have been identified in only around 37% of the examined species (Fig. 1). The AFLP method, which dates back to the mid-1990s, is also rarely used (Vos et al., 1995). Despite its high efficiency (a high level of polymorphism: 50-100 bands per primer per individual) (Vos et al., 1995), the popularity of AFLP decreased gradually due to high cost, and multiple and time consuming analytical phases. ISSR is a simple and reproducible method that overcomes many of the limitations noted in RAPD and AFLP, but to date, it has been rarely used to determine sex markers in plants (it was used in only 17% of the analyzed species) (Fig. 1). RAPD and ISSR primers generate sequences with a limited number of bands, therefore, polymorphism analyses that rely on the above methods can be laborious. Without the luck factor, dozens or even hundreds of primers need to be tested. To illustrate - only 10 RAPD primers had to be analyzed in *Cycas circinalis* to find differences in band sequences between male and female individuals (Gangopadhyay et al., 2007), whereas *Asparagus officinalis* required examinations of 760 primers (Jiang and Sink, 1997).

What makes sex determination easier?

In a review of studies into sex determination in plants, Ainsworth (2000) noted that most researchers identified sex-linked markers for males. This trend has been visibly reversed in the past 11 years, and the number of species where markers have been found for both sexes continues to increase (Fig. 2). In some cases, several markers are identified by the same research team with the application of the same method, and a study of *Sphaerocarpos* testifies to the above (McLetchie and Collins, 2001).

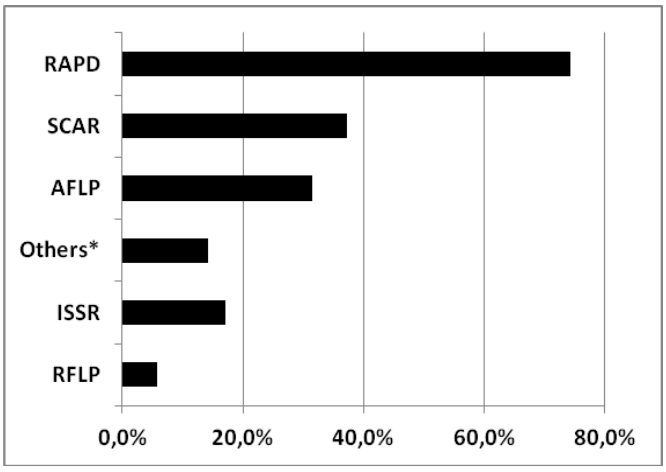

Fig 1. The most popular methods of sex determination in plants based on the species analyzed in this study (frequency of application in percentage) *mentioned in Table 1

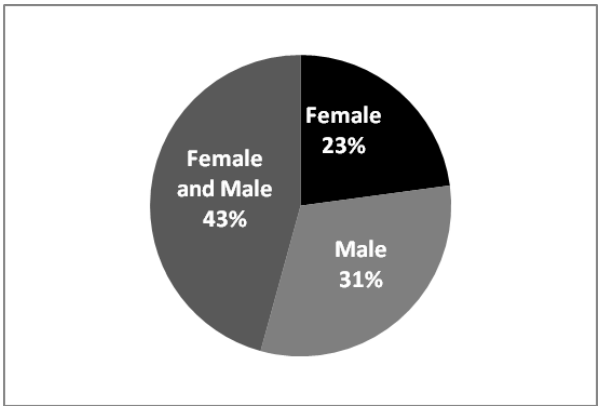

Fig 2. The number of plant species where sex-linked markers have been found for males, females, both males and females (based on the species analyzed in this study).

In other experiments, including a study of *Phoenix dactylifera* (Younis et al., 2008), the same group of scientists may use different marker systems to observe genetic differences between sexes. There are still different studies in which separate teams complement their achievements finding markers specific for each sex of the investigated species. Such was the case with *Carica papaya* (Parasnis et al., 1999; Gangopadhyay et al., 2007). Sex-linked markers are more likely to be found in cases where a sex chromosome system functions (Ainsworth, 2000; Korpelainen et al., 2008). Since the majority of research studies conducted before 2000 reported male-specific markers, we can assume that males of the investigated species are heterogametic (Ainsworth, 2000), and sex-linked markers are related to maleness chromosomes. Sex-linked markers for both sexes are even easier to identify in haploid plants with a chromosomal mechanism of heredity. The above is clearly manifested in bryophytes. In *Sphaerocarpos texanus*, 3 markers for females and 1 marker for males were identified (McLetchie and Collins, 2001), whereas 2 markers characteristic of females and 6 markers typical of males were observed in *Marchantia polymorpha* (Fujisawa et al., 2001). In haploid plants containing sex chromosomes, there are even chances of determining male and female markers because the haploid genome contains only one sex chromosome: X or Y. When heteromorphic

chromosomes are absent, and sex is determined by genes on autosomal chromosomes, significantly fewer markers are identified in a single species (Ruas et al., 1998).

Searching for sex-linked markers brings additional information

A smaller number of sex-linked markers than the number of primers used in DNA genome screening could also point to the small size of the DNA segment involved in sex determination (Hormaza et al., 1994; Gunter et al., 2003). The above correlation was observed in a study of *Pistacia vera*, where 700 different RAPD primers were used, but only a single female-specific marker was found. It is quite likely that in the above plant, only a single gene is responsible for the sex determination mechanism (Hormaza et al., 1994). It may seem puzzling that using an identical genome screening method, one team can quickly identify the differences between male and female individuals, whereas another group of scientists has to apply hundreds of various primers. Mulcahy et al. (1992) used only 60 RAPD primers to identify 4 markers characteristic of the Y chromosome in *Melandrium album*, whereas McLetchie and Collins (2001) tested 200 RAPD primers to discover the same number of sex-lined markers in *Sphaerocarpos texanus*. The above could result from insignificant differences in X and Y chromosome sequences in this liverwort plant. The study by McLetchie and Collins (2001) points to a homology between the sequence specific for males, and one of the sequences characteristic of females. The similarity is considerable enough to suggest the existence of alternative forms of the same locus in homologous regions of X and Y chromosomes. A greater number of homologous regions on sex chromosomes could exist in *Sphaerocarpos texanus*. Comparisons of male- and female-specific sequences in a species can provide valuable information about the organization of regions responsible for sex determination. Absence of homology generally indicates that sequences originated from different chromosome regions, but when the degree similarity reaches 80%, sequences could also be derived from different loci (Khadka et al., 2002). The higher the number of non-homologous sequences, the larger the region which is responsible for sex determination. The degree of homology between male- and female-specific sequences also supplies information about the chromosomes on which they are located. Korpelainen et al. (2008) suggested that at 40% similarity, such sequences are unlikely to represent the genetic material of heteromorphic sex chromosomes. Nonetheless, such a possibility cannot be ruled out. Heteromorphic sex chromosomes could have similar or even partially shared regions (Korpelainen et al., 2008). An example of the above is *Marchantia polymorpha*, where at least 6 of 64 Y chromosome genes have analogs on the X chromosome (Yamato et al., 2007). Additionally, the degree of variability (the number of substitutions) in homologous regions could indicate whether the analyzed sequences originate from coding regions. A high number of substitutions in homologous regions could suggest that those sequences originated in non-coding regions with accumulated mutations (Korpelainen et al., 2008).

Mistakes in sex determination

The ideal sex determination method should produce reliable results and be relatively easy to perform. When a marker for only one of the sexes has been found, there is a risk that analysis results were false. The absence of a PCR product in the sample could be a negative result, but it could also explain the absence of DNA in the sample or other inconsistencies in the PCR process. Ideally, a researcher should use one or two different SCAR markers which create products of different length in males and females in the same amplification. Sex-linked markers for *Ginkgo biloba* were determined in line with the above method. SCAR markers generated products with the length of 571 bp for males and 688 bp for females (Liao et al., 2009). Annealing temperature differed for both primer pairs, but various temperatures for this amplification stage can be set in the thermocycler. Situations such as those encountered in the study of *Ginkgo biloba* happen rarely. Even if the markers of both sexes are found in the same species, they are rarely discovered by the same research team, and their identification is a laborious process. The male-specific marker of *Asparagus officinalis* was found in 1997 (Jiang and Sink), whereas the female-specific marker of the taxon was identified only a decade later (Gao et al., 2007). When a sequence characterized marker is developed for only one species, researchers make various attempts to guarantee the reliability of their findings. Tests of primer pairs for male-specific sequences in *Melandrium album* (Zhang et al., 1998) and *Mercuralis annua* (Khadka et al., 2002) revealed pairs which generated products not only in males but also amplicons of different length in females and monoecious individuals (Khadka et al., 2002). The internal PCR control is an alternative method which was successfully used by Parasnis et al. (2000) and Stehlik and Blattner (2004). Parasnis et al. (2000) used the chloroplast DNA intergenic spacer sequence in a study of *Carica papaya*, whereas Stehlik and Blattner (2004) relied on the internal transcribed spacer (ITS2) sequence in analyses of *Rumex nivalis*.

Conclusions

Despite the growing body of research, the mechanism of sex determination in many plant species remains unexplained. The search for molecular sex-linked markers paves the way for future scientific discoveries. Sex-linked markers alone do not explain the molecular mechanism of sex determination in dioecious plants, but the number of markers, their sequence structure and homology between sequences characteristic of males and females provide a certain venture point for studies into sex determination mechanisms. Sex differentiation in plants is a popular research topic, and the results of recent studies complement previous findings. In pre-2000 studies, the majority of discovered markers were characteristic of males, whereas more recent research efforts found markers for both sexes of the same species. Attempts are made to identify sex-linked markers not only in cultivated plants, as illustrated by the study of bryophytes where both male and female markers were discovered. Such achievements could stimulate new population studies analyzing the proportions of the sexes in dioecious plants where sexual dimorphism is weakly expressed.

Acknowledgements

Monika Milewicz is a scholarship holder in the project „Dr INNO 3. Doctoral scholarships" organized by Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn and funded by European Union within the European Social Fund.

References

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

Ainsworth Ch (2000) Boys and girls come out to play: The molecular biology of dioecious plants. Ann Bot- London 86: 211- 221

Allen CA (1917) A chromosome difference correlated with sex differences in *Sphaerocarpus*. Science 46: 466- 467

Alström-Rapaport C, Lascoux M, Wang YC, Roberts G, Tuskan GA (1998) Identification of RAPD marker linked to sex determination in the basket willow (*Salix viminalis* L.). J Hered 89: 44-49

Banerjee NS, Manoj P, Das MR (1999) Male-sex-associated RAPD markers in *Piper longum* L. Curr Sci India 77: 693-695

Biffi R, Restivo FM, Tassi F (1995) A restriction fragment length polymorphism probe for early diagnosis of gender in *Asparagus officinalis* L. Hortic Sci 30(7): 1463-1464

Bischler H (1986) *Marchantia polymorpha* L. S. LAT. Karyotype analysis. J Hattori Bot Lab 60: 105- 117

Brown TA (2001) Słowniczek. In: Brown TA (ed) Genomy, Wydawnictwo Naukowe PWN, Warszawa

Danilova TV, Karlov GI (2006) Application of inter simple sequence repeat (ISSR) polymorphism for detection of sex-specific molecular markers in hop (*Humulus lupulus* L.). Euphytica 151: 15- 21

Di Stilio VS, Kesseli RV, Mulcahy DL (1998) A pseudoautosomal random amplified polymorphic DNA marker for the sex chromosomes of *Silene dioica*. Genetics 149: 2057-2062

Eppley SM, Maureen LS, Grosberg RK (1998) Intrapopulation sex ratio variation in the salt grass *Distichlis spicata*. Am Nat 152(5): 659-670

Fujisawa M, Hayashi K, Nishio T, Bando T, Okada S, Yamato KT, Fukuzawa H, Ohyama K (2001) Isolation of X and Y chromosome- specific DNA markers from a liverwort, *Marchantia polymorpha*, by Representational Difference Analysis. Genetics 159: 981-985

Gangopadhyay G, Roy SK, Ghose K, Poddar R, Bandyopadhyay T, Basu D, Mukherjee KK (2007) Sex detection of *Carica papaya* and *Cycas circinalis* in pre-flowering stage by ISSR and RAPD. Curr Sci India 92(4): 524- 526

Gao WJ, Li RL, Li ShF, Deng ChL, Li SP (2007) Identification of two markers linked to the sex locus in dioecious *Asparagus officinalis* plants. Russ J Plant Physl + 54(6): 816- 821

George J, Karun A, Manimekalai R, Rajesh MK, Remya P (2007) Identification of RAPD markers linked to sex determination in palmyrah (*Borassus flabellifer* L.). Curr Sci India 93(8): 1075-1077

Gunter LE, Roberts GT, Lee K, Larimer FW, Tuskan GA (2003) The development of two flanking SCAR markers linked to a sex determination locus in *Salix viminalis* L. J Hered 94(2): 185-189

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

Hormaza JI, 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

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

Khadka DK, Nejidat A, Tal M, Golan-Goldhirsh A (2002) DNA markers for sex: molecular evidence for gender dimorphism in dioecious *Mercurialis annua* L. Mol Breeding 9: 251-257

Korpelainen H, Bisang I, Hedenäs L, Kolehmainen J (2008) The first sex-specific molecular marker discovered in the moss *Pseudocalliergon trifarium*. J Hered 99(6): 581-587

Kumar S, Singh BD, Sinha DP, Rai M (2008) Sex expression-associated RAPD markers in pointed gourd (*Trichosanthes dioica*). Pitrat M. (editor): Cucurbitaceae *2*008. Paper presented at the Proceedings of the IXth EUKARPIA meeting on genetics and breeding of Cucurbitaceae. Avignon (France), 21-24 May 2008, pp.543- 550

Liao L, Liu J, Dai Y, Li Q, Xie M, Chen Q, Yin H, Qiu G, Liu X (2009) Development and application of SCAR markers for sex identification in the dioecious species *Ginkgo biloba* L. Euphytica 169: 49-55

Mandolino G, Carboni A, Forapani S, Faeti V, Ranalli P (1999) Identification of DNA markers linked to the male sex in dioecious hemp (*Cannabis sativa* L.). Theor Appl Genet 98: 86- 92

Manoj P, Banerjee NS, Ravichandran P (2005) Development of sex-associated SCAR markers in *Piper longum* L. PGR Newsletter 141: 44-50

Manoj P, Banerjee NS, Ravichandran P (2005- 2008) Development of sex specific moleculars markers in dioecious *Piper longum* L. plants by differential display. JATIT 459- 465

McLetchie DN, Collins AL (2001) Identification of DNA regions specific to the X and Y chromosomes in *Sphaerocarpos texanus*. Bryologist 104: 543-547

Milewicz M, Sawicki j (2011) Molecular identification of sex in dioecious moss *Nyholmiella obtusifolia* (*Orthotrichaceae*) on the basis of ISSR markers. Čas Slez Muz Opava (A) 60: 1-6

Mulcahy DL, Weeden NF, Kesseli R, Carroll SB (1992) DNA probes for the Y-chromosome of *Silene latifolia*, a dioecious angiosperm. Sex Plant Reprod 5: 86-88

Mwase WF, Erik-Lid S, BjØrnstad Å, Stedje B, Kwapata MB, Bokosi JM (2007) Application of amplified fragment length polymorphism (AFLPs) for detection of sex-specific markers in dioecious *Uapaca kirkiana* Muell. Årg. Afr J Biotechnol 6(2): 137-142

Paran I, Michelmore R (1993) Development of reliable PCR based markers linked to Downy mildew resistance genes in lettuce. Theor Appl Genet 85: 985- 993

Parasnis AS, Ramakrishna W, Chowdari KV, Gupta VS, Ranjekar PK (1999) Microsatellite $(GATA)_n$ reveals sex-specific differences in papaya. Theor Appl Genet 99: 1047-1052

Parasnis AS, Gupta VS, Tamhankar SA, Ranjekar PK (2000) A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings. Mol Breeding 6: 337- 344

Parker, JS (1990) Sex chromosomes and sexual differentiation in flowering plants. Chromosomes T 10: 187- 198

Polley A, Steigner E., Ganal MW (1997) Identification of sex in hop (*Humulus lupulus*) using molecular markers. Genome 40: 357-361

Persson HA, Nybom H (1998) Genetic sex determination and RAPD marker segregation in the dioecious species sea buckthorn (*Hippophae rhamnoides* L.). Hereditas 129: 45-51

Prakash S, Staden J (2006) Sex identification in *Encephalartos natalensis* (Dyer and Verdoorn) using RAPD markers. Euphytica 152: 197-200

Rahman MA, Ainsworth CC (2004) AFLP analysis of genome difference between male and females in dioecious plant *Rumex acetosa*. J Biol Sci 4(2): 160-169

Ramsay HP (1966) *The Hepaticae* and *Anthocerotae* of North America. Volume I: 1-802, Columbia University Press, New York

Renganayaki K, Jessup RW, Burson BL, Hussey MA, Read JC (2005) Identification of male-specific AFLP markersin dioecious texas bluegrass. Crop Sci 45: 2529-2539

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

Ruas CF, Fairbanks DJ, Evans RP, Stutz HC, Anderesen WF, Ruas PM (1998) Male- specific DNA in the dioecious species *Atriplex garrettii* (*Chenopodiaceae*). Am J Bot 85(2): 162- 167

Ruiz Rejón C, Jamilena M, Garrido Ramos M, Parker JS, Ruiz Rejón M (1994) Cytogenetic and molecular analysis of the multiple sex chromosome system of *Rumex acetosa*. Heredity 72: 209- 215

Sakamoto K, Shimomura K, Komeda Y, Kamada H, Satoh S (1995) A male- associated DNA sequence In dioecious plant, *Cannabis sativa* L. Plant Cell Physiol 36(8): 1549-1554

Samantaray S, Geetha KA, Hidayath KP, Maiti S (2010) Identification of RAPD markers linked to sex determination in guggal [*Cammiphora wightii* (Arnott.)] Bhandari. Plant Biotechnol Rep 4: 95-99

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

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

Sharma A, Zinta G, Rana S, Shirko P (2010) Molecular identification of sex in *Hippophae rhamnoides* L using isozyme and RAPD markers. The China Study 12(2): 62-66

Shaw AJ (2000) Mating systems in bryophytes. In: Shaw AJ, Goffinet B (ed), Bryophyte Biology, Cambridge University Press 377- 380

Shibata F, Hizume M, Kuroki Y (1999) Chromosome painting of Y chromosome and isolation of a Y chromosome-specific repetitive sequence in the dioecious plant *Rumex acetosa*. Chromosoma 108: 266-270

Shibu MP, Ravishankar KV, Anand L, Ganeshaiah KN, Shaanker U (2000) Identification of sex- specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). PGR Newsletter 121: 59- 61

Shirkot P, Sharma DR, Mohopatra T (2002) Molecular identification of sex in *Actinidia deliciosa var. deliciosa* by RAPD markers. Sci Hortic- Amsterdam 94: 33- 39

Spada A, Caporali E, Marziani G, Portaluppi P, Restivo FM, Tassi F, Falavigna F (1998) A genetic map of *Asparagus officinalis* based on integrated RFLP, RAPD and AFLP molecular markers. Theor Appl Genet 97: 1083-1089

Stehlik I, Blattner FR (2004) Sex-specific SCAR markers in the dioecious plant *Rumex nivalis* (*Polygonaceae*) and implications for the evolution of sex chromosomes. Theor Appl Genet 108: 238- 242

Sztuba- Solińska J (2005) Molecular markers systems and their application in plant breeding. Kosmos 54 (2-3): 227-239

Terauchi R, Günter K (1999) Sex determination in *Dioscorea tokoro*, a wild yam species. In: Ainsworth C (ed) Sex determination in plants. BIOS Scientific Publishers Ltd, Oxford: 165-174

Tracey LP, Koelewijn HP, Dijk PJ (2004) Identification of a male- specific AFLP marker in a functionally dioecious fig, *Ficus fulva* Reinw. ex Bl. (*Moraceae*). Sex Plant Reprod 17: 17-22

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407- 4414

Wang XM, Song WQ, Liu S, Li XL, Chen RY (2001) AFLP markers related to sex in dioecious plant, *Ginkgo biloba* L. Acta Scientiarum Naturalium Universitatis Nankaiensis 34(1): 5-9

Wang D-W, Li Y, Li Z-Q (2011) Identification of a male-specific amplified fragment length polymorphism (AFLP) and a sequence characterized amplified region (SCAR) marker in *Eucommia ulmoides* Oliv. Int J Mol Sci 12: 857-864

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531- 6535

Xu W-J, Wang B-W, Cui K-M (2004) RAPD and SCAR markers linked to sex determination in *Eucommia ulmoides* Oliv. Euphytica 136: 233- 238

Yamato KT, Ishizaki K, Fujisawa M, Okada S, Nakayama S, Fujishita M, Bando H, Yodoya K, Hajashi K, Bando T et al. (2007) Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. P Natl Acad Sci USA 104: 6472- 6477

Yang Z, El Aidi J, Ait-Ali T, Augur C, Teller G, Schoentgen F, Durand R, Durand B (1998) Sex-specific marker and *trans*-zeatin ribosidase in female annual mercury. Plant Sci 139: 93-103

Younis RAA, Ismail OM, Soliman SS (2008) Identification of sex- specific DNA markers for date palm (*Phoenix dactylifera* L.) using RAPD and ISSR techniques. Res J Agric & Biol Sci 4(4): 278- 284

Zhang YH, Di Stilio VS, Rehman F, Avery A, Mulcahy D, Kesseli R (1998) Y chromosome specific markes and the evolution of dioecy in the genus *Silene*. Genome 41: 141-147