CHAPTER 2

REVIEW OF LITERATURE

2.1 IDENTIFYING MARKERS LINKED TO SEX IN PLANTS

The life cycle of higher plants (angiosperms) is characterized by alternation of generations, during which the sporophyte, the diploid plant body, produces haploid spores (microspores and megaspores) that develop into multicellular haploid gametophytes producing haploid gametes (sperm, egg). Therefore, the terms “male” or “female” do not refer to plants producing “male” or “female” gametes, but to the “stamen” or the “pistil” on the reproductive structure (i.e. flower). The pistil and stamen are the parts of the flower sheltering ovules and producing pollen grains, respectively. Sex determination is traditionally considered to be this selective abortion of the gynoecium or androecium of initially hermaphrodite floral primordia, but it should also be considered to include the differentiation of gametophyte (egg versus sperm) within the pistil or stamen, which occurs in all angiosperm flowers. The flowers of most plant species have stamens and a pistil. Such plants are classified as hermaphrodite plants because they have bisexual flowers. Some plant species, such as cucumber and maize, are classified as monoecious plants, with each plant having both male and female flowers. In such cases, the male flowers have well developed stamens and an undeveloped pistil, while the reverse is true for the female flowers. Moreover, some species, such as Asparagus, Rumex, Humulus, Silene and Cannabis, are classified as dioecious because individual plants have either male or female flowers exclusively. In most of typically dioecious plants, pistil and stamen develop on separate individuals, which are distinguished as “pistillate plants” and “staminate plants”. Such sex separation (dioecy) is found in some 15,000 species in 1,300 genera and 60 families (Parker, 1990). Although the separation of male and female sex organs in different individuals (dioecy) is a widespread mechanism that guarantees recombination and genetic diversity promoted by outcrossing in the animal kingdom, dioecious plants only account for 6% of flowering species (Renner and Ricklesfs, 1995).

Dioecy (separate male and female individuals) is well established in animals, but occurs sporadically in plants. Charlesworth (1985) identified 1303 genera and 170 families in which
dioecy is well established, understanding the genetic and epigenetic factors controlling sex determination in dioecious plants is still at a preliminary stage. Sex determination in dioecious plants may often be genetic or environmental, and only a tiny fraction of them has evolved sex chromosomes. Genetic sex determination may be due to a single locus, multiple tightly linked loci on autosomes, multiple unlinked loci on autosomes, or several genes located on heteromorphic chromosomes (Parrish et al., 2004).

Dioecious plant species have arisen separately during the evolution of angiosperms, and have therefore adopted different mechanisms to control sexual dimorphism, from species in which sex is controlled by either a single gene or a number of non-linked genes to species in which sex determination depends on sex chromosomes (Ainsworth et al., 1998; Jamilena et al., 2008). Sex chromosomes have been found in a small number of species belonging to fifteen families (Charlesworth and Guttman, 1999; Matsunaga and Kawano, 2001; Vyskot and Hobza, 2004; Ming et al., 2007). Most of them are homomorphic and cannot be distinguished in size and shape from each other or from autosomes, and only few species belonging to five families, Hepaticae, Cannabidaceae, Caryophillaceae, Cucurbitaceae and Polygonaceae, have distinctive sex chromosomes as heteromorphic as those found in mammals and insects. The sex chromosomes of plants are therefore much younger than those of animals, but the mechanisms that regulate sex determination in plants are quite similar to those of animals. In some species, such as Silene latifolia and Carica papaya, sexual dimorphisms are controlled by an active Y chromosome, the Y being necessary to activate male development, as in mammals. In others species, such as Rumex acetosa and Cannab sativa, sex determination is not dependent on the presence or absence of the Y chromosomes, but is controlled, as in Drosophila and Caenorhabditis elegans, by the balance between the number of X’s and autosomes (X/A balance).

Early sex identification in plants is important for breeders. Female individuals are generally considered to be more valuable in agriculture as they produce seeds and fruit. Species whose female representatives are more desirable in the production process include Actinidia deliciosa (Shirkot et al., 2002), Carica papaya (Parasnis et al., 2000), Eucommia ulmoides (Xu et al., 2004; Wang et al., 2011), Hippophae rhamnoides (Persson and Nybom, 1998; Sharma et al., 2010), Piper longum (Manoj et al., 2005), Pistacia vera (Hormaza et al., 1994; Kafkas et al.,
2001; Ehsanpour et al., 2008; Esfandiyari et al., 2012), *Phoenix dactylifera* (Younis et al., 2008; Elmeer and Mattat, 2012; Dhawan et al., 2013; Mohamed and Sami, 2015) and *Simmondsia chinensis* (Agrawal et al., 2007; Sharma et al., 2008; Agarwal et al., 2011). In other species, male individuals are of a greater breeding value, among them *Asparagus officinalis* whose male individuals produce larger and thicker shoots (Reamon-Büttner et al. 1998; Reamon-Büttner and Jung, 2000; Gao et al., 2007; Kim et al., 2014). 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. It also helps in planning breeding programs where there is gender bias in plant. 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.

Tool supporting sex determination in plants gave rise to studies investigating different methods to study sex. In plants the bio-/chemo-diversity due to gender is over and above the diversity observed within a species due to location, seasons, developmental stages, etc. In nature it is possible to have a gender-associated qualitative or quantitative difference which may ultimately impact the levels of metabolites and hence drug yield/recovery and efficacy of that taxon. Gender distinction in *P. betle* in vegetative state can be made using Direct Analysis in Real Time Mass Spectroscopy (DARTMS), a robust high throughput method (Bajpai et al., 2012). But commonly used biochemical markers (isozymes/allozyme) are not reliable for gender differentiation. The main weakness of allozymes is their relatively low abundance and low level of polymorphism. In addition, their selective neutrality may be in question (Berry and Kreitman, 1993; Hudson et al., 1994; Krieger and Ross, 2002). Most of the studies thus focus on identifying molecular markers for gender differentiation. 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 analysed 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.
2.1.1 Dioecious plants and identified molecular marker systems for sex determination

*Actinidia chinensis* - The genus *Actinidia*, generally known as golden kiwi fruit consists of about 60 species of woody dioecious vines which have originated in China and neighbouring countries. *Actinidia chinensis* Planch. is a species containing both diploid and tetraploid races. It is closely related to the hexaploid cultivated species *A. deliciosa*. All species in the genus *Actinidia* are functionally dioecious and there is no way to distinguish sex of seedlings on base of cytological or vegetative features. Plants reach reproductive maturity in 3-5 years, with male plants making up approximately 50% of breeding populations (Testolin *et al*., 1995). In populations where fruiting selections are desired, the requirement to grow seedlings to maturity before eliminating the unwanted males represents a considerable cost in plant maintenance. A method for identifying the sex of seedlings would therefore increase the efficiency of a breeding programme by permitting the early elimination of unwanted plants.

Random primers (500) were screened in female and male bulk-DNA preparations derived from siblings to identify two sex-linked RAPD markers in *A. chinensis*. One marker, SmX, was inherited by female progeny from the male parent, and the other, SmY, was inherited by male progeny from the male parent. Inheritance studies of the sex-linked RAPD markers have demonstrated that an XY-type system of sex determination functions in *Actinidia* with the male being the heterogametic sex (Harvey *et al*., 1997). Gill *et al*., (1998) converted RAPD markers SmX and SmY into sequence-characterised amplified regions (SCARs) for the large-scale screening of *Actinidia* breeding populations. These two sex-linked SCAR primers were also reported to function with plants from some other geographically separate accessions of *A. chinensis* and with plants in the closely related polyploid species *A. deliciosa*, but did not amplify a sex-linked band in more distantly related species of *Actinidia*. Zhang *et al*., (2015) identified three sex-specific simple sequence repeats (SSR) markers which can be used to accurately identify sex type male and female kiwifruit.

*Actinidia deliciosa* - *Actinidia deliciosa* (fuzzy kiwifruit) belongs to the genus Actinidia which consists of dioecious vines distributed on the Asian continent. It is considered native to China, since most of the species are in the southwest of the country. Other species of the genus are found in Taiwan, Malaysia, Korea, Japan, Nepal, India, Vietnam and Russia. *Actinidia deliciosa*
is intensely cultivated all over the world. *Actinidia deliciosa*. Hayward (kiwifruit) is a major fruit crop that is cultivated extensively in New Zealand, Italy, Chile, Greece, and other temperate to sub-tropical areas. The kiwifruit industry is based on a single cultivar so, the need for new cultivars has been recognised and breeding programmes have been established. Species of *Actinidia* other than *A. deliciosa* have economic potential, and diversity in characters such as fruit colour, flavour, skin texture, fruit size and vine growth habit offer the opportunity for improvement and variety through selection and interspecific hybridisation (Harvey *et al*., 1995). Many species bear edible fruit but it is *A. deliciosa* var. *deliciosa* which has become most popular for commercial cultivation. Identification of male and female genotypes and of different cultivars within the species is the first step towards the correct classification of kiwifruit germplasm. Breeding programmes have been initiated to develop new cultivars, but dioecy represents an important constraint in kiwifruit breeding programmes and also requires identification of male and female genotypes before planting an orchard.

Mc Neilage (1997) have reported that *A. deliciosa* is a hexaploid, i.e. \(2n = 6x = 174\). Females are XXXXXXX and males are XXXXXXY. The X and Y chromosomes are not morphologically distinguishable in Actinidia due to their small size. It is the presence of a single Y system that allows retention of dioecism. Shirkot *et al*., (2002) studied 34 random primers to identify sex in kiwifruit and identified eight sex-linked markers. Six were female-specific and two were male-specific in nature.

*Asparagus officinalis*- The genus Asparagus comprises three subgenera Asparagus, Protasparagus and Myrsiphyllum which include 100–300 species distributed in the arid and subarid regions of Europe, Asia, Africa and Australia. The species of the first subgenera are dioecious, with unisexual flowers. Asparagus (*Asparagus officinalis*) plants are naturally dioecious, either male or female, with \(2n = 2x = 20\) chromosomes and a haploid genome size of 1323 Mb (Bennett and Leitch, 2003). Sex determinants are located on the L5 homomorphic chromosome pair (Loptien, 1979). Female plants are homozygous for the recessive alleles \((mm)\), while male plants are either homozygous \((MM)\) or heterozygous \((Mm)\) at the sex locus. Andromonoecious plants in asparagus populations are heterozygous for the \(M\) gene and are able to produce some seeds after self-pollination. Male plants are superior to females for important characters such as longevity, growth precocity, productivity and are more tolerant to diseases
(Benson, 1982). For breeding, $MM$ males are preferred as pollinators because they produce all male hybrid populations following crossing with females. However, the $MM$ males cannot be differentiated from the $Mm$ males even at the flowering stage. Presently, time-consuming testcrosses must be performed to screen supermale ($MM$) plants. In addition, to distinguish male and female plants, seedlings must be grown until flowering which generally takes 2 to 3 years. Early induction of flowering is possible but not without difficulties (Abe et al., 1990). Therefore, it is highly necessary to develop codominant sex-linked molecular markers in order to distinguish male, female, and supermale plants at the early seedling stage and to speed-up the breeding process. This would also benefit the cloning of sex-determining genes and the understanding of corresponding mechanism at the molecular level.

A RAPD marker that mapped 1.6 cM to the sex locus, $M$, has been converted into a sequence-characterized amplified region (SCAR) marker (Jiang and Sink, 1997). Biffti et al., (1995) reported a restriction fragment length polymorphism probe for early gender diagnosis in $A. officinalis$. One male and one female specific marker were identified. Gao et al., (2007) tested one hundred decamer primers of random-amplified polymorphic DNA on dioecious $A. officinalis$ plants to identify sex-linked molecular markers. One primer (S368) produced two markers (S368-928 and S368-1178) in female plants. These two DNA markers were identified in 30 male and female plants, respectively, and a S368-928 marker was reported to be linked to the female sex locus. The female-linked S368-928 marker was sequenced and specific primers were synthesized to generate a 928 bp marker of sequence characterized amplified regions (SCAR) in female plants, SCAR 928. SCAR 928 was reported to be used to correctly screen homozygous $mm$ female plants of $A. officinalis$.

Reamon-Büttner et al., (1998) identified nine AFLP markers linked to the sex locus in $A. officinalis$ L. by nonradioactive AFLP technique and bulked segregant analysis. Three of the nine AFLP markers did not give recombinants in three different populations. These markers, namely: E31M56, E41M50 and E33M53 are, therefore, potential universal markers in screening for female and male plants in asparagus populations. With codominant scoring of the markers, they could be utilized in distinguishing the heterozygote (XY) males from the homozygote (YY) ones.
Reamon-Büttner and Jung, (2000) developed five STS markers from AFLP markers to identify sex in *Asparagus officinalis* L. AFLP markers STS4150.1, STS4150.2, STS4150.3 and STS3156 did not give recombinants in five different populations. These markers were male specific and could distinguish YY and XY male plants from female plants. A sex-linked random amplified polymorphic DNA (RAPD) marker was identified from *A. officinalis* L. and was converted into a sequence-characterized amplified regions (SCAR) marker (Kim et al., 2014).

*Calamus simplicifolius*- It is a dioecious rattan species endemic to China. Rattans (canes) are spiny palms comprising approximately 600 species in 13 genera of sub-family Calamoideae, family Palmae. They occur naturally in tropical and subtropical Asia as well as equatorial Africa and represent very important non-wood forest products worldwide, only next to bamboos. Over exploitation of rattan has led to a decrease of this natural resource. To meet the domestic demands for rattan materials, plantations have been established. Under most circumstances, seeds for these plantations are highly mixed ones, traditionally collected from natural forests or other plantations, and the resulting plants fail to produce adequate high quality canes. Therefore, it is necessary to develop a rattan breeding program for improved seed quality. Establishing a seed orchard is a good way in which this could be achieved. However, *C. simplicifolius* is a dioecious plant and may be similar to other species of dioecious rattans; wherein male represent a higher proportion of plantation plants than do females (Alloysis, 1997). This represents an impediment to seed orchard establishment because only a few male plants are needed to ensure the fertilization of female plants. Also, the sex of the plant cannot be determined with morphological characters till the reproductive maturity age of 5 or more years, which remains a problem for early sex diagnosis. In an improvement program, as a consequence, this prompts remarkable constraints for a priori mating design of superior individuals in breeding practices and for sex allocation of seedlings in seed orchard establishment. Species in the genus Calamus are usually diploid with 2n = 2x = 26 chromosomes (Sarkar and Datta, 1985; Roser, 1994; Wang et al., 2005), despite 2n = 2x = 28 in exceptional case in *C. palustris* Griff. (Indira and Anto, 2002). There is no evidence recorded of any sex chromosomes in the genus.

In *C. simplicifolius*, efforts have been made to identify molecular markers linked to sex determination. A male-linked random amplified polymorphic DNA (RAPD) marker has been reported in above mentioned plant species (Yang et al., 2005).
Li et al., (2010) developed a male-specific SCAR marker (CsMale1) in the dioecious rattan species *Calamus simplicifolius*. The marker was 509 bp in size and had a GC content of 50.1%. The sequence contained two open reading frames, indicating that CsMale1 represented probably a coding genome region.

**Carica papaya** - Papaya (*Carica papaya*) belongs to the small family Caricaceae with six genera and 35 species, of which 32 are dioecious, two trioecious, and one monoecious. The papaya plant (*Carica papaya* L.) is cultivated mainly in tropical countries where its fruit, the papaya, is very important not only as a food but also economically and socially due to its high acceptance in the international market. The consumption of papaya is growing as the fruit is low in calories and sodium, but high in dietary fibre, calcium, potassium and vitamins A and C (Parasnis et al., 1999; Gamage et al., 2003).

Papaya (*Carica papaya* L.) is a polygamous species with three sex types: male, hermaphrodite and female. In most cases, hermaphrodite plants are preferred for commercial use. For commercial planting hermaphrodite plants from the Solo group of papayas are preferred because they produce pear-shaped fruits which have thicker flesh and a smaller internal cavity than fruits from female plants (Marin and Gomes, 1987). Solo group hermaphrodite plants are usually propagated from seeds from plants with desirable characteristics, the seeds giving rise to uniform progeny if obtained from a known cultivar, although this results in hermaphrodite and female plants at a ratio of 2:1 (Hofmeyr, 1938; Storey, 1938). Since the use of seeds produce seedlings of unknown sex expression, producers have to plant seedlings in groups of three and thin out the female and male plants after 3 to 4 months when it is possible to identify the sex of the seedlings from their floral buds (Lemos et al., 2002). This process increases production costs and makes the final product more expensive to the consumer. And the loss to the farmer being greater the larger the cultivated area and the longer the time the female plants compete for water, light and nutrients with the hermaphrodites (Marin and Gomes, 1987). Effort has been made over recent years to obtain a method for the early detection of the sex of seedlings before they are planted in the field. DNA and isozyme analysis of the genus *Carica* are being undertaken by several research groups to study sexual determination in *C. Papaya*. 
Sondur et al., (1996) constructed a genetic linkage map for *C. papaya* based on random amplified polymorphic DNA (RAPD) markers and investigated the genetics of sex determination in papaya, detecting the existence of a sex-linked locus (SEX1) segregating in the proportion of 2 hermaphrodites to 1 female with lethality of the dominant homozygote (SEX1-H/SEX1-H).

Andreani Jr. and Lemos, (1995) have reported the existence of a peroxidase isozyme biochemical marker for male sex in papaya, but isozymes present some limitations as biochemical markers because they show polymorphism due to post-translation modifications, environmental conditions and stage of development of the seedling (Soltis and Soltis, 1989). On the other hand, DNA molecular markers are not subject to these limitations and appear to be the best option for genetic analysis.

Parasnis et al., (1999) reported a microsatellite sequence (GATA)$_4$ unique to males or hermaphrodites of several cultivars. Additionally, Parasnis, (2000) and Urasaki et al., (2002) reported SCAR markers that are specific for male and hermaphrodite plants. Deputy et al., (2002) identified papaya male and hermaphrodite sex linked RAPD and SCAR markers. The developed molecular markers were reported to be strongly linked to Sex1, the gene that determines plant sex in papaya (*Carica papaya* L.).

Lemos et al., (2002) reported a RAPD based molecular marker which was able to detect hermaphrodites in all the tested cultivars.

A 10-mer Operon primer, OP-Y7 (5'-AGAGCCGTCA-3') was identified (Chaves-Bedoya and Nuñez, 2006). This RAPD marker of 900 bp was found in male plants, but not in females or hermaphrodites. From this RAPD marker a sequence characterized amplified region (SCAR) was developed and it was possible to amplify fragments from the genomes of male and hermaphrodite plants, but not the female ones. Gangopadhaya et al., (2007) reported a female and hermaphrodite specific ISSR marker in *C. Papaya*. Niroshini et al., (2008) reported a RAPD based SCAR marker which showed sex specificity to male and hermaphrodite plants. In order to obtain simple DNA markers to identify sex expression in papaya, Sobir et al., (2008) utilized five SCAR markers of 20–21 primers. Examination of these markers into 24 genotypes of papaya from 12 populations of different genetic background revealed that pair of primer PKBT-5 had successfully differentiated male and hermaphrodite plants from female plants.
Reddy *et al.*, (2012) reported identification of molecular and morphological markers like leaf markers and rate of growth at juvenile/seedling stage for different sex types and their evaluation at flowering stage in five varieties of papaya. It was found that based on the leaf morphology and rate of growth, male and female seedlings could be identified at seedling stage. The study indicated that the seedlings start with single lobed leaves and differentiates into three and five lobed leaves. The three lobed leaves are predominant in males and five lobed leaves in females. Leaf morphology and rate of growth at seedling stage has been exploited in identifying the presumptive males and female seedlings at juvenile seedling stage (60-75 cm height). The above results were confirmed through RAPD (Randomly Amplified Polymorphic DNA) for different sex types and at flowering stage in field. A PCR based SCAR marker was reported for sex determination in papaya by Chaturvedi *et al.*, (2014).

*Commiphora wightii*- Guggul [*Commiphora wightii* (Arnot.) Bhandari] belongs to the family Burseraceae and is a well-known drug plant. Its exudates are pharmacologically active in controlling rheumatoid arthritis, obesity and peptic ulcer, and its oleoresin also acts as diaphoretic expectorant, diuretic and emmenagogue. It has significance in the treatment of anti-inflammatory, antirheumatic, hypocholesterolemic, hypolipidemic, and antifertility activities (Kakrani, 1981). Moreover, a hypolipidemic drug from guggul gum resin containing guggul sterones along with other lipids has been developed and marketed and is in use in the allopathic, Ayurvedic and Unani systems to combat human ailments (Kapoor *et al.*, 1979; Nityanand and Kapoor, 1971; Taj-ud-din *et al.*, 1994). Africa and Asia are the centres of origin of the genus *Commiphora*, and *C. wightii* is distributed in the arid tracts of the Rajasthan and Gujarat states of India and the Sind and Baluchistan provinces of Pakistan. The species faces extinction due to faulty extraction methods employed by traditional resin collectors. As a result, there has been a significant decline in the number of guggul plants available for extraction of drug mainly due to the death of the mature plants. The entire demand of oleo–gum–resin in pharmaceutical industry has been met from tapping of wild growing plants for centuries. Dioecy and bisexual plants have been reported in *C. wightii*. It is not possible to discriminate between them until flowering, which takes about 1–2 years. However, there are also no phenotypic characters available to discriminate the male, female, and hermaphrodite plants. Understanding the molecular basis of sex expression has immense importance both in basic and applied research.
Samantaray et al. (2009) tested decamer RAPD primers on dioecious and hermaphrodite plants of Commiphora wightii to identify sex-specific molecular markers. Three markers were identified which consisted of one for female, one for hermaphrodite and one for both female and hermaphrodite.

Cannabis sativa- Cannabis sativa is an annual herbaceous plant in the Cannabis genus, a species of the Cannabaceae family. Cannabis sativa L. is a dioecious plant with two heteromorphic sex chromosomes. The genus Cannabis is one of the most ancient cultivated crops utilized for fibre production and drug preparation and more recently for oil extraction from the seeds and for paper manufacturing. From a caryological point of view all hemp forms are reported to have a 2n = 20 chromosome set and are supposed to have male heterogamety, though the latter is still controversial (Yamada, 1943; Mohan Ram and Sett, 1985). Yamada, (1943) revealed that the female plants have two X chromosomes whereas the male plants have one X chromosome and one Y chromosome, with the latter being much larger than the X chromosome and the autosomes.

Usually, the hemp (Cannabis sativa L.) cultivars employed have been typically dioecious, but monoecious cultivars have been developed as well. Monoecious hemp is particularly useful when both seed production and stem harvesting is performed; the main disadvantages in the use of monoecious hemp essentially lie in the occurrence of self-pollination, causing a lower stem yield, and in the slower rate of genetic progress attainable compared to dioecious hemp (Bócsa, 1994). Besides, only in dioecious hemp can the Bredemann principle be applied, which consists in the scoring of the fibre content in male plants and the subsequent pollination of only those plants with the highest fibre content (Bredemann, 1938). As the adoption of this method implies a direct identification of the male plants, which is possible only when reproductive maturity approaches, the risks of contamination resulting in seeds obtained by undesired pollinations is not negligible. For these reasons it would be very useful in hemp genetic improvement programs to be able to achieve an early recognition of male or female plants, consequently anticipating the fibre content analysis well before the flowering time or designing the crosses to be performed early during the vegetative phase of the plants.
Sakamoto et al., (1995) analyzed male-associated DNA sequences in Cannabis sativa L. (family: Moraceae). A 730bp DNA fragment was identified with the help of RAPD primers which produced intense male specific bands and was named MADC1 (male-associated DNA sequence in Cannabis sativa). Mandolino et al., (1999) also reported a male specific 400-bp RAPD marker generated by a primer of random decamer sequence and subsequently sequenced and converted it into a SCAR marker. Törjék et al., (2002) reported two male specific molecular markers (MADC5 and MADC6) in hemp (Cannabis sativa). Sakamoto et al., (2005) reported that accumulation of retrotransposable elements on the Y chromosome might be one cause of heteromorphism of sex chromosomes. They reported that RAPD markers encoding retrotransposable elements are linked to the male sex in Cannabis sativa L.

Encephalartos natalensis - The Cycads are the oldest group of plants surviving on Earth. All the living cycads are included in the order Cycadales. Among the genera, Encephalartos is the second largest among the genera. Encephalartos species are widely distributed across various climatic zones of the African continent. All Encephalartos species are strictly dioecious with 2n = 18 chromosome numbers. There is no recorded evidence of any monoecious genotypes. The plants of two sexes are morphologically alike and sexuality of the plants only becomes clear once the first cones appear. There is no way to predict sex in the cycads at the juvenile stage (Whitelock, 2002).

Prakash and Staden, (2006) reported development of a sex specific Random Amplified Polymorphic DNA (RAPD) marker for Encephalartos natalensis. Initially, 140 primers were used to amplify the bulk DNA of five individuals each of known male and female sexuality. While a high degree of polymorphism was observed in the amplification profiles of male and female plants, only primer OPD-20 generated a specific band (~850 bp) in female DNA.

Ginkgo biloba - Ginkgo biloba, a dioecious perennial gymnosperm, is the only living representative of the order Ginkgoales left in the tertiary (65 million years ago). It is therefore called living fossil. The maidenhair tree Ginkgo biloba is a widely popular deciduous and dioecious gymnosperm species that is native to China and has been cultivated for well over a millennium. In Asia, G. biloba is used in traditional medicine and its seeds, also known as Ginkgo nuts, are considered a delicacy and are also a popular cuisine item. Male trees are used
for landscaping. Female trees produce seeds that emit a noxious, foul odour on falling to the ground and therefore female trees are considered to be undesirable. The slippery pulp can also be a liability. These features caused city governments to actually remove and ban the female from being planted. Male ginkgoes do not produce a fruit and are selected as the main cultivars used to transplant in urban communities. This male cultivar of Ginkgo casts dense shade and the dense crown makes it suitable as a screen or noise buffer. The time when young Ginkgo trees begin to flower intervenes between 15 to 25 years of growth in nature. In addition, it is not very easy to distinguish the fertile shoot from the common vegetative spur shoot before reproductive time. Therefore, sex identification in the early stages of plant life is of great importance in the strategy of plant management and utilization.

Ling et al., (2003) reported a marker specific for male plants in Ginkgo trees collected from Beijing and Shenyang. Echenard et al., (2008) reported development of an automated random polymorphic DNA analysis (ARPA), a new automated technology which proved highly effective in distinguishing males and females with 100% efficiency and was reported to be successful in male and female discrimination from a collection of young seedlings derived from a sexual cross. Marker S1478 was validated for sex determination in Ginkgo trees from two Chinese regions. For early sexual determinism in G. Biloba an automated version of RAPD was developed, which previously relied on manual agarose gel electrophoresis and DNA detection by ethidium bromide staining. Automated random polymorphic DNA analysis (ARPA) claims to have several advantages over the former method, thus increasing its usefulness as a technique for assessing the sexual determinism in G. biloba. A capillary electrophoresis system was used to resolve large (up to 682 bp) ARPA–polymerase chain reaction size fragments from plant genomic DNA, representing a new application for this automated system. The results obtained with ARPA should be cautiously interpreted. As a molecular technique that relies on total DNA extraction and PCR amplification, ARPA is subject to the usual problems associated with DNA quality for performance in PCR.

Liao et al., (2009) reported identification of a male and a female specific marker in G. biloba using the RAPD marker system. These two above mentioned markers were further converted into male and female specific SCAR markers. Wang et al., (2001) reported identification of three female and one male specific AFLP polymorphism.
Seabuckthorn (Hippophae rhamnoides L.) is a multipurpose tree belonging to the family Elaeagnaceae, with six species and twelve subspecies in the world. In Asia, seabuckthorn is distributed through the Himalayan regions including India, Nepal, Bhutan and Northern parts of Pakistan and Afghanistan. In Himachal Pradesh, Hippophae species are found in the valleys of Laddakh, Lahaul and Spiti and Kinnaur districts. It has rich medicinal properties and plays an important role in environmental conservation, in afforestation programs in hill regions. Recently, it has gained importance as a horticultural tree for berry production because of its high nutritional and medicinal properties. The berries are acidic in nature and the most prominent feature of its juice is its rich source of vitamins C, E and K. Nutritious products from seabuckthorn include tea, juice, wine, jams and snacks. The medicinal properties include prevention of cardiovascular diseases, cancers, skin problems, burns and digestive tract disorders and for anti-senilism, anti-inflammation, anti-radiation damage and for improvement of the immune system.

H. rhamnoides L. is a diploid species with a basic chromosome number 2n = 24, of which eleven pairs are autosomes and one pair consists of sex chromosomes (Lebeda, 2003). It’s dioecious in nature and the sex of saplings cannot be determined until flower buds appear which may take 5–7 years. For economic reasons, the ratio of male to female plants is important in fruit bearing plants, i.e., their pistillate trees should be optimum and a proportion of 8–12% of staminate trees is considered adequate for pollination (Walf and Wegart, 1993). Singh, (1998) has recommended a 9:1 ratio of female to male trees for seabuckthorn. Thus, correct identification of staminate and pistillate genotypes at its juvenile phase is important to maintain proper densities of pistillate and staminate bushes/trees in a plantation.

Study was carried out by Sharma et al., (2010) to develop male and female specific isozymes and RAPD markers so that the gender of H. rhamnoides L. can be identified at its juvenile stage and material can be raised as staminate and pistillate populations. The isozyme analysis was conducted with four enzyme systems, viz. peroxidase, esterase, malate dehydrogenase and catalase. The peroxidise enzyme system produced a female specific sex marker, which successfully differentiated between the staminate and pistillate genotypes of H. rhamnoides L. Thirty five random decamer primers were also studied and one male sex linked marker was identified, OPD-20 (5’-ACTTCGCCAC-3’) which displayed a band at 911 bp that expressed
polymorphism between male and female genotypes. The staminate and pistillate genotypes could be distinguished using RAPD marker OPD-20\textsubscript{b11}. Korekar \textit{et al.}, (2012) identified and validated two female specific SCAR markers in dioecious \textit{Hippophae rhamnoides L.}

\textit{Humulus lupulus}- Hop (\textit{Humulus lupulus}) is a cultivated plant in the family Cannabaceae that is mainly used in the production of beer and to some minor extent for pharmaceutical purposes. In beer production, unfertilized female flowers or their extracts are the major contributors of the aromatic bitter taste and simultaneously serve as a preservative and foaming agent.

Hop is a dioecious perennial plant. For the beer production, only vegetatively propagated female plants are grown. Genetically, cultivated European hop is diploid. Sex specificity in Japanese hop is determined by the ratio of the number of X chromosomes to the number of autosomal sets (A), i.e., in line with genetic balance theory. The sex of hop plants can only be reliably determined at the time of flowering, which is 1-2 years after the planting of seeds from a cross. For breeding and selection of new hop lines, it would be highly desirable to have a method for rapid identification of female plants at the seedling stage, so that male and female plants could be separated at that time.


To develop a sex-specific marker in Japanese hop (\textit{Humulus japonicus} Siebold & Zucc.), Aleksandrov \textit{et al.}, (2011) analyzed 36 ISSR markers on the basis of pools of male and female plants identified after flowering. ISSR marker K-16 was identified, which manifested stable amplification of an approximately 300bp fragment in male plants and the absence of amplification in female plants in the populations examined.
**Mercurialis annua** - *Mercurialis annua* is an annual dioecious commonly known as annual mercury. It belongs to the family Euphorbiaceae. It does not have sex chromosomes (Durand and Durand, 1991). It has a multi-loci mechanism for gender determination: three unlinked loci $A$, $B_1$ and $B_2$ are involved in gender determination (Louis, 1989). Multiple genotypes resulting from the combinations of these loci are possible in both males and females. Cytokinin and auxin are involved in sex expression, the former being feminizing and the later masculinising hormones (Durand and Durand, 1994). Males can be classified into three subtypes - strong, intermediate and weak, on the basis of their resistance to feminization by cytokinins.

Dioecious *Mercurialis annua* is diploid ($2n = 16$). Its polyplid relatives show variation in sexuality depending upon the ploidy level and populations. Tetraploids are strictly monoecious while hexaploids are monoecious but may also be androdioecious or gynodioecious. The octaploids and dodecaploids are strictly monoecious (Durand and Durand, 1990; Pannell, 1997).

A female specific PCR marker was reported from dioecious *Mercurialis* using the AP-PCR technique (Yang et al., 1998). Khadka et al., (2002) reported identification of two male specific Random Amplified Polymorphic DNA (RAPD) markers, OPB01-1562 and OPC07-303, in dioecious *Mercurialis annua* and designing of Sequence Characterized Amplified Region (SCAR) primer.

**Momordica dioica** - *Momordica dioica* Roxb. commonly known as “spine gourd” or “teasle gourd,” belongs to the family Cucurbitaceae. This is a perennial, rhizomatous, distinctly dioecious climber found in the forests of Southern India and Bengal. Many species of the genus *Momordica* exist in the wild in India and the surrounding geographical region in South and Central Asia, indicating that the centre of origin of *M. dioica* might be in this region. *Momordica dioica* is an important vegetable with high food value and contains good amount of carotene and protein. Tender fruits and tuberous roots are used as vegetable and in ayurvedic medicine. The medicinal properties of this plant are sex-specific and each sex has its own medicinal value. The leaves of female *M. dioica* are used as an aphrodisiac, to eradicate intestinal parasites, and to cure fever, asthma, hiccups, and piles. The root of female *M. dioica* is used in the form of a medicinal paste to heal bleeding piles, also used with benefit in headaches, kidney stones, and jaundice. The fruit is considered pungent, bitter, hot, alexiteric, stomachic, and laxative, and
plays a role in cures for biliousness, asthma, leprosy, bronchitis, fever, tumors, urinary discharges, excessive salivation, and heart disease. The root of male *M. dioica* is used in the form of a paste to heal ulcers caused by snake bites. It is also useful to cure elephantiasis. The male plant is bitter, pungent, hot, with wound-healing properties, and it targets diseases of the blood, eyes, and heart. Karyomorphological studies (Baratakke and Patil, 2009) reveal that male and female *M. dioica* exhibit no morphological differentiating markers and possess homomorphic chromosomes which make sex screening nearly impossible.

Baratakke and Patil, (2009) reported identification of a male specific marker in *M. Dioica* using the RAPD technique. Patil *et al.*, (2012) developed a male specific RAPD based SCAR marker for sex identification at the pre-flowering stage in *M. Dioica*. A total of 50 random decamer primers were used for screening of specific RAPD markers in male and female populations. Only one primer, OPA-15, amplified genomic DNA in different patterns in the male versus female genotypes. This sex-specific band OPA-15\textsubscript{1500} was cloned and sequenced. Based on the RAPD sequence, a pair of SCAR primers MSSM-01F and MSSM-01R was developed. These SCAR primers amplified a single 1501 bp DNA band only in male populations. This band was subsequently named SDSM (Sequence Discriminating Sex of *Momordica dioica*). Baratakke *et al.*, (2013) reported a molecular marker based on Random Amplification of Polymorphic DNA to identify female plants before flowering stage with reference to medicinal values.

**Pandanus tectorius**- *Pandanus tectorius* Parkinson (= *P. fascicularis* Lam.), locally called ‘Kewda’ or ‘Kia’, belongs to the Pandanaceae and is a wild and dominant perennial species in the coastal vegetation of Orissa. Traditionally, the plant was used for fencing agricultural fields to protect them from cattle grazing. The plant grows profusely near the seashore in xeric conditions, mainly along the coast, providing ecological benefits such as arresting soil erosion and forming the basis of sand dunes, thus acting as a natural bulwark against wind. This plant is dioecious, with distinct male and female inflorescences on different plants. The inflorescence of the male plant constitutes the raw material from which the aroma ‘rhu’ or essential oil (phenyl ethyl methyl ether, terpine-4-ol, p-cymene, a-terpineol, etc.) is extracted (Panda *et al.*, 2007, 2009). Male plants support the flourishing perfume industry that is presently confined to Ganjam District along the coast of Orissa. Generally, female inflorescences (and fruits) are of little use.
The plant is vegetatively propagated, and sex is only clear after 5–7 years when plants from stem cuttings start to flower.

Panda et al., (2010) reported two male specific RAPD markers in *Pandanus tectorius* Parkinson for early determination of sex. Early determination of the sex of the plant was also examined through analysis of somatic chromosome compliment, genomic DNA content and random amplified polymorphic DNA (RAPD) analysis in seven populations of *P. tectorius* from the coast of Orissa. Whereas the chromosome complement (2n=60) failed to reveal any differences, the 4C DNA amount indicated that the genome of female plants [6.15 pg = 5935 mega base pair (Mbp)] was significantly larger than that of male plants (5.09 pg = 4912 Mbp).

**Phoenix dactylifera**- Date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated trees. Palms (Arecaceae) display great diversity in their reproductive morphology, with more than 85% of the palm genera having single sex flowers. The date fruit produced largely in the hot arid regions of South West Asia and North Africa, is marketed all over the world as a high-value confectionery and fruit crop and remains an extremely important subsistence crop in most of the desert regions. The establishment of a date palm plantation is a long term and costly investment. Offspring from seeds are approximately equally split between males and females; however, only the female trees produce the fruit that is sought after in farming. Dioecy presents a challenge in date palm breeding programs because it is impossible to distinguish trees until they flower approximately five to eight years after planting. In all date palm, a major problem for farmers is to identify the sex of saplings at an early stage so that they can cultivate in their orchards a sufficiently large number of productive female trees with only a minimal number of male trees.

Younis et al., (2008) identified sex-specific DNA markers for some date palm (*Phoenix dactylifera* L.) cultivars using molecular technique (RAPD and ISSR) to facilitate the selection and identification of good male pollinators for further utilization in breeding programs to increase the yield and to improve some quality traits of fruits. They reported three positive specific markers for females and two for males were reported in RAPD analysis. Also five positive specific markers were reported for males in ISSR analysis.

Elmeer and Mattat, (2012) reported a male specific marker in date palm. Using 14 microsatellite primer pairs with 129 date palm leaves and tissue culture samples from 34 cultivars which
represent the major date palm diversity of Qatar, 254 microsatellite loci were detected, of these, 22 microsatellite loci could be used to identify 9 out of 12 male date palm samples (75%). The data also indicated that the heterozygous allele with the size 160/190 produced by the primer mPdCIR048 reoccurred 4 times exclusively in the 12 individual male samples but not in any of the 117 female date palm samples tested. Moghaieb et al., (2010) identified 13 female specific and 5 male specific RAPD markers in date palm. These markers could be applied to screen male and female plants at young stages. Dhawan et al., (2013) reported development of male-specific SCAR marker in date palm.

**Piper betle**- Betel vine (*Piper betle* L.) belongs to family Piperaceae and is an economically important cash crop valued for its fresh leaves in many parts of India. Betel leaf is closely associated with cultural traditions of India and is considered as a holy plant. The green, heart shaped leaves of this plant are masticatory and popularly known as *paan* in India. It has many medicinal usages in Indian system of medicines to cure indigestion, stomachache, diarrhoea, flatulence and to heal wounds, bruises, swellings due to sprains, bruises, respiratory disorders, constipations, boils and gum disorders. Studies also revealed that the leaf improves immune system and inhibits cancer growth. Roots are known for female contraceptive effects. Both male and female clones are cultivated based on local preference. Sex determination in this crop is not readily possible as flowering in both the sexes is observed in specific regions of the country (Northeast and Western Ghats, India). Since sexual dimorphism is associated with economic traits (leaf length and width), great improvement in leaf yield and quality in the crop could be achieved through heterosis breeding (Maiti *et al*., 1992) which has been already initiated to develop new cultivars. Though phenotypic characters like leaf shape are available to identify the male and female plants in the absence of flowering (Maiti and Biswas, 1991), however, it does not reflect conspicuously at early seedling stage. However, since the inability to identify gender at early ages can create problems in advanced-generation breeding programmes, particularly when all superior parental selections or all progeny are unknowingly composed of one gender.

Samantaray *et al*., (2012) used the Random Amplified Polymorphic DNA (RAPD) technique to amplify DNA segments, with the objective of finding markers linked to sex determination in male and female plants of *Piper betle* L. Fifty different random decamer primers were screened to identify markers associated with sex expression of which only four primers were found to be
associated with sex expression. These four primers were then tested with individual plant DNA samples where sex-associated RAPD markers were identified. Three male specific RAPD markers OPA041400, OPA08650 and OPN02850 and two female-specific markers OPA081200 and OPC06980 were reported which could reliably differentiate the male and female plants of *P. betle* L.

Khadke *et al.*, (2012) studied 35 ISSR primers in 20 germplasm lines of *Piper betle* L. Two primers, viz. ISSR-10 and UBC-852 produced male specific bands of size 459 bp and 1250 bp respectively. ISSR-23 amplified a female-specific 636 bp fragment. A sequence characterized amplified region (SCAR) from the primer ISSR-23 was also developed, which amplified fragments from the genomes of females, but not the male ones.

**Piper longum**- Long pepper (*Piper longum*), (Pippali), sometimes called Indian long pepper, is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Male and female plants differ in the morphology of their spikes, which bear minute achlamydous unisexual flowers. Mature female spikes, known, as 'long Pepper' is shorter and thicker than the male spikes. *Piper longum* plants are functionally dioecious and have no distinguishing cytological or vegetative features to identify the sex of plants. In populations where flowering selections are desired, the requirement to grow plants to maturity before eliminating the unwanted males represents a considerable loss in plant maintenance.

Manoj *et al.*, (2005) analyzed genetic differences in a germplasm collection consisting of 96 female and 40 male accessions of *P. longum* L. bearing spikes. The purified genomic DNA was subjected to PCR for RAPD analysis using random decamer oligonucleotide primers (OPA1-20, OPE1-20, OPD1-20 and OPAC1-20). RAPD profiles of the male samples generated by OPA10 and OPA15 primers were distinctly different from those of the females because of the presence of prominent male-associated bands OPA10827 and OPA15744. A male specific RAPD based SCAR marker was also constructed.

**Pistacia vera**- The genus *Pistacia* is a member of the Anacardiaceae family and consists of at least eleven species. *P. vera* has edible nuts and is commercially important. *Pistacia vera* L. (pistachio) is dioecious species, is an important crop tree and produces commercially valuable nuts. It reaches reproductive maturity after 5–8 years. In commercial orchards, the problem of
gender ratio is overcome by grafting a scion of known gender on the rootstock. However, in breeding programs and forestation, distinguishing male from female individuals at an early stage of development would be advantageous.

Kafkas et al., (2001) studied Random Amplified Polymorphic DNA (RAPD) markers linked to sex in *P. atlantica*, *P. terebinthus* and *P. eurocarpa*, the major wild species in Turkey used as rootstocks for *P. vera*. Hormaza et al., (1994) detected a single marker associated with females and absent in males in *Pistacia vera* L. using RAPD markers.

Yakubov et al., (2005) reported that a combined method of sequence characterized amplified regions (SCAR) primers and Touchdown-PCR was used for the development of a female DNA marker in *Pistacia vera* L. The random decamer primer OPO-08 amplified a 905-bp fragment in all female trees, but also in several males. SCAR primers designed on the basis of the RAPD female molecular marker amplified a 905-bp female and a 909-bp male fragment. Ehsanpour et al., (2008) studied gender of four female and male individual *Pistacia vera* cultivars (Akbari, Ahmad Aghaai, Fandoghi, Kaleh Ghochi) using nine Inter Simple Sequence Repeat (ISSR) primers. Two primers (AC)$_8$CG and (AC)$_8$TA were able to identify male and female plants by producing sex dependent DNA bands in female plants. Esfandiyari et al., (2012) reported the development of a female specific RAPD based SCAR marker.

*Poa arachnifera* Torr.- *Poa* is a large, diverse genus that belongs to the sub family Pooideae of the family Poaceae. Several species are complex polyploids with a range of breeding systems, including apomixis and dioecy, that promote interspecific hybridization and introgression. Because of this, it is frequently difficult to taxonomically distinguish one species from another. Texas bluegrass ($2n = 8x = 56$) is a dioecious, perennial cool-season grass that is native to southern Kansas, Oklahoma, western Arkansas, and most of Texas. It is drought and heat tolerant and produces high quality forage in regions where other cool-season temperate grasses, such as tall fescue (*Festuca arundinacea* Schreb.), are not sustainable. It produces only limited quantities of seed that are covered with woolly hairs which are difficult to remove. Consequently, establishment of stands for agricultural use is difficult. Although little molecular information is available regarding Texas bluegrass, a study using AFLP markers investigated the diversity within the species and determined that genetic mapping is feasible (Renganayaki et al., 2001).
The molecular characterization of dioecy in Texas bluegrass could provide valuable tools for plant breeders working with cereal, forage, and turf grasses.

Renganayaki et al., (2005) constructed AFLP based linkage maps for both the paternal and maternal plants. Two male specific AFLP markers were reported.

*Rumex acetosa*- The dioecious plant species ‘Sorrel’ (*Rumex acetosa* L.) has evolved a distinctive sex chromosome system with 2n=12+XX in females and 2n=12+XY_1Y_2 in males. Sex is determined by ratio between the number of X-chromosome and number of autosome sets. The major sex determining genes locate on the X-chromosome. While Y-chromosomes contain genes for pollen fertility and play important role in sex chromosome distribution in male meiosis, they are not required for sex expression. Sorrel is a unique model plant for the study of sexual dimorphism, use of molecular markers to analyse the difference between male and female genomes will help in understanding the origin of Y-chromosomes.

Rahman and Ainsworth, (2004) identified four AFLP based male specific markers. Two male associated DNA fragments were isolated and sequenced them. It was asserted that generation of very few male specific AFLP markers and their sequence similarity to the stretches of human DNA from autosomes and both the X and Y-chromosomes indicate the likely origin of the Ys from the X-chromosome.

*Rumex nivalis*—Rumex nivalis (commonly called Snow dock) is a species of flowering plant belonging to family Polygonaceae. It’s a perennial herb and is well defined by its small size, mostly unbranched inflorescence, a basal rosette, and stems with no or rarely up to two fleshy leaves. The species is dioecious with 2n = 14 for females and 2n = 15 for males. *Rumex nivalis* grows on calcareous, wet soils in snowbeds or along creeks mainly above the timberline at 1600–2900 m above sea level. It displays a female biased sex ratio.

Stehlik and Blattner, (2004) developed SCAR primers based on isolated and sequenced male-specific fragments as identified in an AFLP analysis of the dioecious plant *Rumex nivalis*. PCR amplification using these primers on females and males resulted in fragments exclusively present in males. The genetic distances between the Y-chromosomal sequences of *R. nivalis* and *R. acetosa*, both members of the section Acetosa, were substantial.
**Salix viminalis** - *Salix viminalis* belongs to dioecious Salicaceae family (willows, poplars), the mechanisms of sex determination have not been resolved, though McLetchie and Tuskan, (1994) reported no evidence of sex chromosomes in *Populus trichocarpa* X *P. deltoides* hybrids, despite testing a large number of potentially linked markers. There is no robust evidence of sex chromosomes in Salicaceae, yet both species are represented by fairly stable sexually dimorphic systems not readily influenced by plant growth substances, although some level of hermaphroditic and female-biased sex ratios has been reported for both *Salix* and *Populus* spp. It has been hypothesized that female-biased sex ratios in *Salix viminalis* are a result of familial relationships rather than abiotic agents, and observed sex ratios in certain genetic backgrounds suggest a multilocus epistatic model of sex determination in *S. viminalis* (Alström-Rapaport et al., 1998). These results could have strong implications for breeding strategies for the development of *S. viminalis* as a short-rotation energy crop.

Alström-Rapaport et al., (1998) studied 380 arbitrary decamer primers to generate randomly amplified polymorphic DNA (RAPD) products. Of the 1080 RAPD bands examined, only a single 560 bp band was shown to be linked to a sex determination locus. A single female specific marker, UBC354560 was reported.

Gunter et al., (2003) identified two RAPD markers that were present in the common female parent as well as in predominantly female progeny of these families. These RAPD markers were sequenced and converted to sequence characterized amplified region (SCAR) markers.

**Simmondsia chinensis** - Jojoba (*Simmondsia chinensis*) a dioecious species, is an important crop shrub that produces commercially valuable seeds in female plants. It is now the only species belonging to the genus *Simmondsia* in family of Simmondsiaceae. It is a shrub of arid zones, has emerged as a cash crop in India and abroad. It is a native of Sonoran deserts of south-western United States of America, north-western Mexico and Baja California. Its seeds stores liquid wax which is used in cosmetic, pharmaceutical and plastic industries. It has promising physical properties, such as high viscosity index, high flash and fire points, high dielectric constant and high stability and freezing point that can be used in various industries. Due to its potential to make canopy and eliminating windy erosion in desert regions in Iran, its adaptation was initiated by Shahsavand et al., (2006). In jojoba sex chromosomes are not distinguishable. Therefore, sex
type of jojoba seedlings cannot be determined by cytological methods (Parasnis et al., 2000). Also, sex type of jojoba seedlings cannot be determined either by embryo shape or morphology at the juvenile developmental stage. Propagation of jojoba is mainly through seeds. Therefore, three to four years are required for this shrub to reach the flowering stage of its life cycle and it is a slow growing and the ratio of male to female plants in the field is around 5:1. In general, male plants are not useful commercially, therefore, the farmers eliminate a considerable number of male plants and this increases production costs. In the Indian climate, one male plant is enough to pollinate five female plants. Therefore, it is of immense agricultural importance to identify the sex of this species at the juvenile stage.

Agrawal et al., (2007) generated a male specific random amplified polymorphic DNA (RAPD) marker in jojoba which is based on the PCR amplification of random locations in the genome of plant. Mohasseb et al., (2009) reported two male specific RAPD markers and one female specific RAPD marker. PCR-based sex determination in jojoba by Sry and Random Primers were also reported. Hosseini et al., (2011) identified one male specific and one female specific marker in Jojoba (Simmondsia chinensis (Link) Schneider) using the RAPD based marker system.

Agarwal et al., (2011) developed sex-linked AFLP markers in Simmondsia chinensis. Two male specific primers and one female specific primer were identified. ISSR marker-assisted selection of male and female plants was reported in jojoba (Simmondsia chinensis) by Sharma et al., (2008). A male specific marker was reported which was absent in all the females. Jangra et al., (2014) reported a male specific SCAR marker in S. chinensis. Heikrujam et al., (2014) generated and validated unique male sex-specific sequence tagged sites (STS) marker from dioecious Jojoba. Two male specific markers were reported using eighty ISSR primers. One of the male-sex specific markers was developed into male-sex specific sequence tagged sites (STS) marker.

**Trichosanthes dioica**- Pointed gourd (Trichosanthes dioica) a genus of family Cucurbitaceae is an annual or perennial, dioecious cucurbit that grows as a vine; it is cultivated in subtropical and tropical regions around the globe. It is propagated through stem cuttings taken, ideally, from mature plants to ensure the sex and fruit types. Although each fruit contains several well-developed seeds having hard seed coat, propagation using seed is not feasible primarily due to poor germination and slow growth of the seed-derived plants. In T. dioica, male and female
plants strictly maintain their respective sexual phenotypes but we are not aware of any report of sex-specific peculiarities in chromosome morphology in this species. Kumar et al., (2007) cultured almost fully developed embryos in vitro to develop a population of 52 sexual progeny, of which 37 were female and only 15 were male, indicating a considerable bias in favour of female plants (female/male ratio, 2.4/1). It was suggested that lethal/sub-lethal gene(s) linked to the female determining locus may be the possible cause of this female bias. Dioecy represents a disadvantage in pointed gourd breeding programmes since, at present, there is hardly any dependable method for distinguishing between male and female plants prior to flowering. A reliable method for determining the gender of plants before flowering would facilitate economy of the various resources, including the time and effort of the breeders. In addition, this would allow the identification and even isolation of the gene(s) involved in the process.

Kumar et al., (2008) extracted DNA samples separately from 10 male and 10 female sexual progeny of pointed gourd (Trichosanthes dioica), and 3 plants from a parthenocarpic clone (IIVRP-105). Forty-one random decamer primers were screened with the three bulks in order to identify markers associated with sex expression. Two RAPD markers, male-specific marker OPC051000 and female-specific marker OPC14400, were identified which can reliably differentiate between male and female plants of T. dioica.

Roy et al., (2008) did a transcript profiling of unopened male and female floral buds of pointed gourd (Trichosanthes dioica Roxb.), a dioecious cucurbit, at uniform development stage through cDNA-AFLP to look for differentially expressed unique and/or upregulated gene fragments associated with sex expression. Thirty one such fragments, twenty three from male and eight from female, were selected, cloned, sequenced and assigned putative protein functions after database searching and obtaining GenBank accessions. The male derived clone (TDM16) was annotated as polygalacturanose with remarkably high level of homology. This protein is reported to have direct involvement with pollen development, germination and tube growth with particular function in depolymerization of pectin.

Kumar et al., (2012) reported one male and one female specific RAPD marker in pointed gourd. Nanda et al., (2013) and Adhikari et al., (2014) also reported ISSR based markers for sex

### 2.2 GENETIC DIVERSITY ANALYSES IN PLANTS

Biodiversity refers to variation within the living world, while genetic diversity represents the heritable variation within and between populations of organisms, and in the context of this literature, among plant species. This pool of genetic variation within an inter-mating population is the basis for selection as well as for plant improvement. Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Diversity in plant genetic resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (yield potential and large seed, etc.) and breeders preferred traits (pest and disease resistance and photosensitivity, etc.). The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. It is particularly useful in the characterization of individual accessions and cultivars for detecting duplications of genetic material in germplasm collections, and for selection of parents for breeding hybrids (Davila *et al.*, 1998). Studies have also revealed that knowledge on genetic diversity and of genetic relationships among breeding materials has a great impact on crop improvement (Ganesh and Thangavelu, 1995).

The assessment of genetic diversity within and between plant populations is routinely performed using various techniques such as (i) morphological, (ii) biochemical characterization/evaluation (allozyme), in the pregenomic era, and (iii) DNA (or molecular) marker analysis in postgenomic era.

Morphological markers are based on visually accessible traits such as flower colour, seed shape, growth habits, and pigmentation, and it does not require expensive technology but large tracts of land area are often required for these field experiments, making it possibly more expensive than molecular assessment in western (developed) countries and equally expensive in Asian and Middle East (developing) countries considering the labour cost and availability. These
marker traits are often susceptible to phenotypic plasticity; conversely, this allows assessment of diversity in the presence of environmental variation which cannot be neglected from the genotypic variation. These types of markers are still having advantage and they are mandatory for distinguishing the adult plants from their genetic contamination in the field, for example, spiny seeds, bristled panicle, and flower/leaf colour variants.

Second type of genetic marker is called biochemical markers, allelic variants of enzymes called isozymes that are detected by electrophoresis and specific staining. Isozyme markers are codominant in nature. They detect diversity at functional gene level and have simple inheritance. It requires only small amounts of plant material for its detection. However, only a limited number of enzymes markers are available and these enzymes are not alone but it has complex structural and special problems; thus, the resolution of genetic diversity is limited to explore.

The third and most widely used genetic marker type is molecular markers, comprising a large variety of DNA molecular markers, which can be employed for analysis of genetic and molecular variation. These markers can detect the variation that arises from deletion, duplication, inversion, and/or insertion in the chromosomes. Such markers themselves do not affect the phenotype of the traits of interest because they are located only near or linked to genes controlling the traits. These markers are inherited both in dominant and codominant patterns. Molecular markers may or may not correlate with phenotypic expression of a genomic trait. They offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell. Additionally, they are not confounded by environmental, pleiotropic, and epistatic effects. A comprehensive study of the molecular genetic variation present in germplasm would be useful for determining whether morphologically based taxonomic classifications reveal patterns of genomic differentiation. This can also provide information on the population structure, allelic richness, and diversity parameters of germplasm to help breeders to use genetic resources with less pre breeding activities for cultivar development more effectively. Germplasm characterization based on molecular markers has gained importance due to the speedy and quality of data generated. DNA markers have numerous applications in plant breeding such as (i) marker assisted evaluation of breeding materials like assessing the level of genetic diversity, parental selection, cultivar identity and assessment of cultivar purity (Winter and Kahl, 1995;
Weising et al., 1995; Baird et al., 1997; Henry, 1997; Djè et al., 2000; Hokanson et al., 2001; Jahufer et al., 2003; Galli et al., 2005; Alvarez et al., 2007; Ali et al., 2008; Becerra et al., 2010; Chandi et al., 2013; Porth and El-Kassaby, 2014; Ammar et al., 2015; Costa et al., 2016; Ramakrishnan et al., 2016), study of heterosis, and identification of genomic regions under selection, (ii) marker assisted backcrossing, and (iii) marker assisted pyramiding (Collard and Mackill, 2008).

Molecular markers may be broadly divided into three classes based on the method of their detection: hybridization-based, polymerase chain reaction (PCR) based, and DNA sequence-based. At present polymerase chain reaction (PCR) based marker systems are more rapid and require less plant material for DNA extraction. Here, PCR based ISSR and AFLP markers are reviewed in context of genetic diversity analysis in plants.

2.2.1 Inter Simple Sequence Repeat (ISSR) Marker in plant genetic diversity

ISSR is a kind of simple and quick technique, permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple repeats, and possesses some advantages of stability and reproducibility, rich polymorphism, reliability (Zietkiewicz et al., 1994 and Gupta et al., 1994), much larger numbers of fragments per primer and relatively low cost, have been widely used for DNA fingerprinting, population genetics and phylogenetic studies and so on in field crops, fruit trees and herbs (Zhou et al., 2007) and was described as a powerful technique to assess genetic diversity (Tian et al., 2008, Wang et al., 2008, Aparajita et al., 2008) and to detect similarities between and within species levels (Ghariani et al., 2003).

Inter-simple sequence repeat (ISSR) has proved to be a highly useful tool for estimating genetic diversity and assessing genetic relationships because it is simple, fast, cost-effective, reliable and highly discriminating (Ci et al., 2008; Crespe et al., 2009; Zhang and Dai, 2010; Uysal et al., 2010; Petros et al., 2008). ISSR have been used to study genetic diversity in Chinese Yam (Dioscorea opposita Thunb) and assess the relationships of 28 cultivars of yam (Zhou et al. 2008). Najaphy et al., (2012) revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Sofalian et al., (2009) showed that ISSR markers could be efficiently used to evaluate genetic variation in
the wheat germplasm. Sofalian et al., (2008) used ISSR markers to determine the genetic diversity of 39 bread wheat accessions, including 33 wheat landraces and 6 wheat cultivars from northwest of Iran. The results indicated high level of polymorphism of wheat landraces based on these markers in contrast to other markers. Cluster analysis suggested that, ISSR markers are efficient tools for estimating intra-specific genetic diversity in wheat and these molecular markers could differentiate the local varieties obtained from different locations. Chowdhury et al., (2008) used ISSRs to fingerprint and estimate genetic diversity in a set of 27 genotypes which comprised Indian bread wheat varieties released for high yield, quality and abiotic stress and trait specific landraces having known pedigrees. It was found that the cluster analysis tree placed these genotypes in six groups and is in agreement with their known origin. The genetic relationships estimated by the polymorphism of ISSR markers revealed greater level of genetic variability in Indian bread wheat varieties of wide adaptability and applicability. Pasqualone et al., (2000) tested the efficiency of ISSR markers to distinguish a set of 30 Italian durum wheat cultivars and 22 breeding lines. Efficiency reported was very high and two primers were sufficient to distinguish all the durum wheat cultivars examined. Abou-Deif et al., (2013) characterized twenty wheat varieties by using ISSR Markers and succeeded in distinguishing most of the 20 varieties in relation to their genetic background and geographical origin. Zamanianfard et al., (2015) evaluated molecular diversity of durum wheat genotypes using ISSR markers.

Genetic diversity among cultivated and wild Chamomilla recutita (L.) Rausch. was evaluated using ISSR markers and it was concluded that they show very high genetic similarity and suggested to find new sources of genetic diversity in chamomile in wild populations (Okoń et al., 2013). ISSR markers have been reported to show differentiation among Italian populations of Asparagus acutifolius L. according to geographical origin (Sica et al., 2005). Ray et al., (2010) evaluated phylogenetic relationship among six economically important species of Asparagus utilizing ISSR polymorphism. Genetic variability was also analysed in ornamental plant Calibrachoa caesia using ISSR markers and used markers allowed the characterization of every individual examined (Pérez de la Torre et al., 2012).

Knowledge of the levels and distribution of genetic diversity is important for designing conservation strategies for threatened and endangered species so as to guarantee sustainable
survival of populations and to preserve their evolutionary potential. *Picconia azorica* is a valuable Azorean endemic species recently classified as endangered. To contribute with information useful for the establishment of conservation programmes, the genetic variability and differentiation among 230 samples from 11 populations collected in three Azorean islands was accessed with eight inter-simple sequence repeat markers (Lopes et al., 2014). A total of 64 polymorphic loci were detected. The majority of genetic variability was found within populations and no genetic structure was detected between populations and between islands. Also the coefficient of genetic differentiation and the level of gene flow indicated that geographical distances do not act as barriers for gene flow. Study also suggested that in order to ensure the survival of populations in situ and ex situ management practices should be considered, including artificial propagation through the use of plant tissue culture techniques, not only for the restoration of habitat but also for the sustainable use of its valuable wood.

Shaw et al., (2009) evaluated genetic diversity among 14 cultivars of medicinal plant *Catharanthus roseus* using ISSR markers. Level of genetic diversity and relatedness among 16 strawberry (*Fragariaxananassa* Duch.) cultivars and 11 breeding lines developed in Canada was analysed using ISSR markers (Debnath et al., 2008). Seventeen primers generated 225 polymorphic ISSR-PCR bands. The ISSR markers detected a sufficient degree of polymorphism to differentiate among strawberry genotypes, making this technology valuable for cultivar identification and for the more efficient choice of parents in current strawberry breeding programs. Tagizad et al., (2010) successfully used ISSR markers for studying genetic diversity of Iranian Pistachio cultivars. Genetic variation among sweet, purple, and yellow passion fruit accessions (*Passiflora alata* and *P. Eduliswas*) were assessed using inter-simple sequence repeat markers (Santos et al., 2011). Eighteen ISSR primers were used to evaluate 45 accessions. Results indicated that ISSR can be useful for genetic diversity studies, to provide practical information for parental selection and to assist breeding and conservation strategies in *Passiflora*. Li and Ge, (2001) investigated genetic variation and clonal diversity of seven *Psammochloa villosa* (Poaceae) populations from northwest China using ISSR markers. They found out that evenness of distribution of genotypes in *P. villosa* populations varied greatly, with all of the genotypes being local ones. No significant differences in genetic or clonal diversity were found between populations in mobile or fixed dunes. The main factor responsible for the high level of differentiation among populations and the low level of diversity within populations
was probably the clonal nature of this species, although selfing might have also affected the population genetic structure to some extent. The efficiency of ISSRs in identifying genetic individuals was much higher than that of allozymes. An approximately asymptotic correlation was found between the number of genets (single genetic individual) detected and the number of polymorphic loci used, suggesting that use of a high number of polymorphic bands is critical in genetic identification.

ISSR markers have been extensively and successfully used in assessing genetic diversity in various aromatic, medicinal, ornamental plants. However, studies involving ISSR markers is lacking in *Piper* sp. Jiang and Liu, (2011) analysed genetic diversity of *Piper* spp. in Hainan Island (China) using repeat ISSR markers. 247 polymorphic bands out of a total of 248 (99.60%) were generated from 74 individual plants of *Piper* spp. The overall level of genetic diversity among *Piper* spp. in Hainan was found to be high. The diversity analysis unambiguously distinguished all *Piper* spp. Sheeja et al., (2013) carried out genetic diversity analysis of 27 *Piper* species using ISSR (Inter Simple Sequence Repeat) markers which indicated that the analysis placed them in six clusters in the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram. The molecular marker based clustering of the species gave supporting evidence to the earlier groupings proposed by the taxonomists using traditional tools. 35 species specific bands were identified from different species. Patra et al., (2011) carried out ISSR marker analysis to establish genetic identity and evaluate genetic diversity among fifteen cultivars of betel vine (*Piper betle*) grown in different parts of Orissa. Results obtained with ISSR markers were compared with those obtained with RAPD markers. All the cultivars were found to be related to each other with an average similarity of 0.2913. Khadke et al., (2015) found ISSR markers as reproducible and specific tools for genetic diversity analysis of betel vine germplasm and *Piper* sp. Genetic diversity analysis in 37 accessions of betel vine and two accessions of *P. hamiltoni* and *P. colubrinum* was carried out using ISSR markers. Out of 60 ISSR primers tested, 15 were selected on basis of high and consistent polymorphism. The UPGMA dendrogram and PCA plot revealed *P. colubrinum* to be the most distant of three species. The accessions from Andaman clustered based on geographical origin and shared 70% similarity. A distinct gender based clustering was also reported among cultivated clones of betel vine.
2.2.2 Amplified Fragment Length Polymorphism (AFLP) Marker in plant genetic diversity

AFLP (amplified fragment length polymorphisms) markers are highly reproducible multi-locus marker system developed by Vos et al., (1995). High levels of polymorphism and high degrees of discriminative capacity are the main advantages of this marker system. The concept of AFLP DNA fingerprinting techniques has the advantage of detecting DNA polymorphism in high resolution, as small as 1 bp. In addition, AFLP techniques have the advantage of being able to apply fingerprinting without sequence information of the genomic DNA (Hulst et al., 2000; Russel et al., 1997), because AFLP fingerprinting relies on primers designed in part on sequences for endonuclease restriction sites, and on two or three selective nucleotides. AFLP analysis detects genetic variation throughout the genome by using a pair of specific restriction enzymes and their corresponding adapters combined with 2 selective rounds of PCR. Because PCR primers are based on the sequences of the restriction enzyme and universal adapters to which they are ligated, the procedure requires no prior information about the nucleotide sequences under investigation. Polymorphism is detected by using a number of selective bases following the restriction site. Primers with one or no selective base are used in a round of pre-amplification. This reaction is diluted for use in a second round of PCR in which primer pairs with 2 or 3 selective bases are used. In addition, it is possible to construct very high density DNA marker maps for application in genome research and positional cloning of genes through this method (Russel et al., 1997; Monte- Corvo et al., 2000).

Moncada and Hinrichsen, (2007) studied genetic diversity among clones of red wine cultivar 'Carmenère' (Vitis vinifera L.) using AFLP markers. 'Carmenère' is a fine red wine cultivar (Vitis vinifera L.) that has spread, unrecorded from France to other countries. Genetic diversity was analysed among 26 accessions from Chile, France and Italy using AFLP markers. Results suggested that 'Carmenère' exhibits a lower genetic diversity in comparison with other French red wine cultivars. Genetic Diversity was analysed in Pongamia [Pongamia pinnata (L) Pierre] using AFLP Markers (Thudi et al., 2010). Pongamia is a leguminous deciduous tree, indigenous to the Indian subcontinent and successfully introduced to humid tropical regions of the world. To assess the molecular genetic diversity in 48 Pongamia pinnata accessions collected from six different states of India, amplified fragment length polymorphism (AFLP) marker system was employed. A good level of genetic diversity was observed in examined germplasm.
and accessions collected from Karnataka showed comparatively higher diversity than accessions from other states. Mukherjee et al., (2006) assessed genetic diversity in thirty one species of mangroves and their associates through AFLP Markers. The percentage of polymorphism detected was too high indicating the high degree of genetic variability in mangrove taxa both at inter- and intra-generic levels. Alfalfa (*Medicago sativa* L.) is a widely grown legume and one of the most important forage species throughout the world. Keivani et al., (2011) did genetic diversity assessment of alfalfa (*Medicago sativa* L.) populations using AFLP markers. Genetic diversity of 26 Iranian cultivated populations of alfalfa (*Medicago sativa* L.) was studied using eight AFLP selective primer combinations. This study provided evidence that AFLP marker is an informative and suitable approach to evaluation of molecular polymorphism and polygenic relationships in cultivated alfalfa (*Medicago sativa* L.). AFLPs have also been successfully used to estimate genetic diversity and relationships among durum wheat accessions (Manifesto et al., 2001; Soleimani et al., 2002; Martos et al., 2005; Medini et al., 2005; Maccaferri et al, 2007; Mardi et al., 2011). Tripathi et al., (2011) assessed genetic diversity among twelve *Aloe vera* accessions using AFLP. Cluster analysis showed considerable level of variability among the collected genotypes. Results provided estimates on level of genetic variation among diverse materials that can be used in assessing the purity and stability of genotypes entering into a breeding or multiplication program.

Shamasbi et al., (2014) evaluated genetic diversity of 17 wild melon (*Cucumis melo var. agrestis*) genotypes in southern Caspian Sea region using AFLP markers. High genetic diversity was observed in the results, which may be attributed to several factors like nature of melon reproduction (cross pollination), high differentiation ability of AFLP markers and existence of several undistinguished subspecies.

Switchgrass (*Panicum virgatum*) is a perennial warm-season grass native to North America that has been identified as a dedicated cellulosic biofuel crop. Todd et al., (2011) quantified genetic diversity in tetraploid switchgrass germplasm collected at Oklahoma State University and characterized genetic relatedness among the collections from distinct regions. Fifty-six tetraploid accessions, including seven upland and 49 lowland genotypes from throughout the US, were examined. The amplified fragment length polymorphism (AFLP) procedure was utilized to generate DNA profiling patterns that were scored visually. Most of the
upland and lowland accessions clustered according to ecotypes. Analysis of molecular variance (AMOVA) was performed which showed significant differences between the upland and lowland genotypes. Garlic (*Allium sativum* L.) is one of the most cultivated vegetables in the world. Outside its centres of origin, garlic propagates only asexually. Since asexual reproduction leads to the absence of meiotic recombination, the main garlic cultivars available for cultivation have arisen from the accumulation of somatic mutations in early cultivars. Thus, it is common for a single clone to have different names in different regions. AFLP markers have been successfully used to study the genetic divergence and identification of garlic clones (Volk *et al*., 2004; Ovesná *et al*., 2007). Morales *et al*., (2013) also used AFLP to identify possible duplicate cultivars in garlic. Genetic diversity among 49 Indian accessions of rice (*Oryza sativa* subsp. *indica*), including 29 landraces from Jeypore, 12 modern cultivars, and 8 traditional cultivars from Tamil Nadu, were investigated using AFLP markers (Prashanth *et al*., 2002). The percentage of polymorphic AFLPs was approximately the same within the cultivars and landraces. Genetic diversity was found to be slightly higher in the modern cultivars than in the traditional cultivars from Tamil Nadu. Study showed that the process of breeding modern cultivars did not appear to cause significant genetic erosion in rice. AFLP has also been used successfully with crops such as tea (Paul *et al*., 1997; Ji *et al*., 2009), almond (Sorkheh *et al*., 2007), barley (Russel *et al*., 1997; Schut *et al*., 1997), Bean (Tohme *et al*., 1996; Fabio *et al*., 2003; Rosales-Serna *et al*., 2005; Kumar *et al*., 2008), Cynodon species (Wu *et al*., 2005), Sugarcane (Lao *et al*., 2009), Evodia (Huang *et al*., 2008), Jujube (Qiao *et al*., 2009), Lentil (Sharma *et al*., 1996), Lettuce (Hill *et al*., 1996), Longan (Peng *et al*., 2008), Naked oat (Xu *et al*., 2009), Potato species (Kardolus *et al*., 1998; Esfahani *et al*., 2009), and has been shown to reveal significant levels of DNA polymorphism.

The pomegranate (*Punica granatum* L.), an ornamental plant which is native to Iran and the Himalayas, produces delicious and edible fruits, and belongs to the *Punicaceae* family. Nemati *et al*., (2012) evaluated the diversity of a number of Iranian pomegranate cultivars using fruit morphological characteristics and AFLP markers. By use of AFLPs, a low genetic diversity level was detected among cultivars. The genetic diversity and genetic relationships of ornamental plant *Osmanthus fragrans* cultivars were analyzed by the technique of AFLP (Yan *et al*., 2009). AFLP markers have successfully been used to study the genetic diversity at the varietal level in
many fruit trees, including apricot (Hurtado et al., 2002), olive (Rotondi et al., 2003) and pear (Bao et al., 2008).

Mueller and Wolfenbarger, (1999) stated that the AFLP markers were effective in the detection of polymorphism among closely related species; AFLP’s have also been used to infer phylogenetic relationships based on measures of genetic distance. Ovesná et al., (2002) reported that the AFLP technology is a powerful tool for the detection and evolution in germplasm collections and in the screening of biodiversity as well as for fingerprinting studies.

In maize, AFLPs have been used to assess’ genetic diversity (Ajmone Marsan et al., 1998; Pejic et al., 1998; Lübberstedt et al., 2000; Yuan et al., 2000). Ajmone Marsan et al., (1998) and Pejic et al., (1998) suggested that AFLPs detect polymorphisms more efficiently in comparison to RFLPs due to larger number of loci assayed in a single PCR reaction. Pejic et al., (1998) found that the easy efficiency index was more than ten-fold higher for AFLPs compared to other marker systems and pointed out that AFLPs can replace RFLPs in genetic similarity studies because of a comparable accuracy in genotyping inbred lines of selected pedigrees. Lübberstedt et al., (2000) also demonstrated that AFLP markers were useful for assigning inbreds into heterotic groups and revealing pedigree relationships among lines.

AFLP markers have been compared with other marker systems in many plants in evaluating genetic diversities. Saker et al., (2005) reported genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. Each type of the three molecular approaches of DNA analysis were reported to identify the different rice genotypes. AFLPs were used to study polymorphism among inbred lines of coffee along with RAPD and SSR marker systems (Maluf et al., 2005). All three marker systems successfully detected polymorphism. Li et al., (2011) compared AFLP and SSR for genetic diversity Analysis of Brassica napus Hybrids and reported AFLP technique was suitable for identification and DNA fingerprinting of B. napus germplasm. Genetic diversity of Brassica napus was also assessed using AFLP, ISSR and SSR markers (Havlíčková et al., 2014). Naghavi and Mardi, (2010) investigated application of AFLPs, and SSRs to examine genetic relationships in the 54 accessions of Aegilops tauschii. Genetic diversity comparison using AFLP and SSR markers have also been reported in bean (Maras et al., 2008) and sweet sorgum (Pecina-Quintero et al., 2012).