Towards Sex Determination of Date Palm

Chapter · January 2011

DOI: 10.1007/978-94-007-1318-5_26

2 authors:

Shawky A. Bekheet
National Research Center, Egypt
62 PUBLICATIONS **360** CITATIONS
SEE PROFILE

Moemen Hanafy
National Research Center, Egypt
26 PUBLICATIONS **164** CITATIONS
SEE PROFILE

Chapter 26
Towards Sex Determination of Date Palm

S.A. Bekheet and M.S. Hanafy

Abstract In the date palm (*Phoenix dactylifera*), a dioecious mode and late initial reproductive age of 5–10 years are major practical constraints to genetic improvement. Improvement of the existing palm cultivars or selection of new ones with superior characters is a tedious endeavor due to the long life cycle of the date palm tree and its heterozygous nature. Sexual propagation method cannot be used commercially for propagating the cultivars of interest in a true-to-type manner. Currently there is no reliable method to identify sex at the early seedling stage. Early sex identification of young seedlings could enhance breeding programs and generate experimental male and female genetic stocks that will help the genetic improvement of the date palm. Moreover, the selection and identification of superior seedling characters for yield enhancement and to improve the physical and chemical properties of fruits is of great commercial interest. There has been significant progress in our understanding of sex-determining mechanisms in date palm using traditional means. But physiological and cytological methods do not give obvious differences between male and female date palms. Biotechnology, as a new tool in date palm breeding, can be useful to improve the qualities of palm trees through early sex identification. Although molecular markers have been introduced in date palm programs, few research efforts have been geared toward studying the early sex determination in the plant. This chapter will focus on genetic and molecular basis of sex determination in date palm in attempting to develop reliable methods to identify sex at an early stage of seedlings.

Keywords Biotechnology • Breeding • Sex determination • Molecular markers

S.A. Bekheet • M.S. Hanafy (✉)
Plant Biotechnology Department, National Research Center, El-Tahrir Str.,
Dokki, 12622, Cairo, Egypt
e-mail: shawky005@yahoo.com; mshanafy@yahoo.com

S.M. Jain et al. (eds.), *Date Palm Biotechnology*,
DOI 10.1007/978-94-007-1318-5_26,

26.1 Introduction

The genetic control of sex determination is well understood in several animal systems, particularly *Drosophila melanogaster*, *Caenorhabditis elegans* and mammals. In plants, understanding of the sex determination system is closely connected with an understanding how separate sexes evolved, and current theoretical ideas about this also illuminate the evolution of sex chromosomes. Angiosperms are of particular interest for empirical studies of sex chromosome evolution because they probably evolved separate sexes repeatedly and relatively recently. Other plants, particularly bryophytes also have interesting independently evolved sex chromosomes (Okada et al. 2001). In many sexually reproducing plant species all individuals are essentially alike in their gender condition. Many such *sexually monomorphic* species are hermaphroditic. The term *cosexual* is used when individual plants have both sex functions, whether present within each flower (hermaphrodite) or in separate male and female flowers (monoecious) (Lloyd and Bawa 1984). A minority of plant species are *sexually polymorphic*, including dioecious species, with separate males and females. Many dioecious species with hermaphrodite relatives have evident rudiments of opposite sex structures in flowers of plants of each sex, suggesting recent evolution of unisexual flowers. The low frequency and scattered taxonomic distribution of dioecy and sex chromosomes suggest that cosexuality is the ancestral angiosperm state (Charlesworth 1985; Renner and Ricklefs 1995). Sex chromosomes therefore probably evolved repeatedly and quite recently.

Sex inheritance and sex chromosomes in plants are strikingly similar to those in animals. Sex-determination systems in plants have evolved independently many times, and are just one of the strategies that promote outcrossing and thus help avoid inbreeding. The majority of plants studied have heterozygous males, or, when the chromosomes are visibly different, perhaps half of plants that have separate sexes, male heterogamety (XY males, XX females). In many dioecious plants, males are *inconstant*, i.e. produce occasional fruits (Lloyd 1975). Self fertilization of such plants in several species has provided genetic evidence that males are heterozygous. The presence or absence of the X chromosome in male gametes provides an efficient mechanism for sex determination.

Knowledge of sex determination in plants indicates that only about 5% of flowering plants, such as hops, date palms and spinach form individuals with separate sexes. Papaya plants can turn out male, female or hermaphroditic. Not all plants with separate sexes have sex chromosomes that look different from their partner. Although economically important, palms are a much neglected plant group in terms of understanding genetics and development potential; therefore much effort must be expended to resolve this problem.

In dioecious plants cultivated for fruit or seed, it is often difficult to identify females at an early stage of growth. Research comparing allocation patterns between genders in dioecious species indicates that female plants usually have higher resource requirements and/or reproduction imposes a greater drain on resources. Perhaps for this reason, a recurring pattern observed among dioecious plant species

is an increasing proportion of males within populations along an axis of decreasing site productivity. Thus early sex identification and genetic characterization of the unknown scattered genotypes of dioecious tree such as date palm resulting from seeds, represent a very important item.

A major problem for farmers is to identify gender 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. Moreover, it is important to select and identify superior males in terms of fertilization. This direct influence of the male parent on the development of the date fruit is precise and definite and varies with the particular male used to fertilize the female flowers. Date palm pollen has been found to exert a direct influence on the size, shape and color of the seed, and also on the size of the fruit, on the speed of development of the fruit and on the time of ripening of the fruit. This direct effect of the pollen on the parts of the seed and fruit lying outside the embryo and endosperm is called *metaxenia*. Date palm breeding is a long-term endeavor (Carpenter 1979). The genetics, morphology, morphogenesis and physiology of date palms are somewhat less understood than other fruit-tree crops. It has been difficult to study because it is native to the subtropics, has a long life cycle and has diverse and unique growth habits compared to other fruit-producing trees. This species is slow flowering and fruiting and it is difficult to determine the sex of the trees before the first flowering, when they are about 5 years of age. Propagation of date palm through seeds or zygotic embryos is desirable for improvement of the cultivars and for selection of diseases resistance, fruit quality and high yield. Also, the early determination of sex type is very important for speeding up breeding programs. Breeding programs to maintain genetic diversity have not been employed because the sex of a date palm cannot be known until it reaches reproductive age (5–10 years) (Bendiab et al. 1993).

Biotechnology, as one of the newest tools in plant breeding, can be helpful for breeders and producers of date palm to improve the qualities through early sex identification. The data suggest that *in vitro* tissue culture conditions can modulate sex modification (Komai et al. 2003), particularly by the reactivation of the arrested organs. Moreover, molecular biology techniques such as Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) can be used to identify sex-specific DNA markers of date palm. With the availability of molecular techniques, we may now hope to understand more about how sex chromosomes evolve. Mapping data, even with anonymous markers, should give estimates of the fraction of X-linked loci that are located in the pairing and differential regions. Once genes have been identified and sequenced, we will be able to estimate how long sex chromosome evolution takes. This should help us to evaluate the plausibility of the proposed mechanisms for the process. In recent years, there have been serious efforts to understand the genetic basis of sex determination in plants and to develop methods to identify sex at an early stage by using molecular marker tools (Biffi et al. 1995; Hormaza et al. 1994; Mulcahy et al. 1992). To achieve progress in understanding sex chromosome evolution and organization in plants, sex-linked genetic markers are required.

26.2 Sex Type of Date Palm

Plant sexuality covers the wide variety of systems found across the plant kingdom. The complexity of the systems and devices used by plants to achieve sexual reproduction has resulted in botanists and evolutionary biologists using numerous terms to describe physical structures and functional strategies. Dellaporta and Calderon-Urrea (1993) list and define a variety of terms used to describe the modes of sexuality at different levels in flowering plants. This list is generalized to fit more than just plants that have flowers, and expanded to include other terms and more complete definitions. Bisexual or perfect flowers have both male (androecium) and female (gynoecium) reproductive structures, including stamens, carpels and an ovary. Flowers that contain both androecium and gynoecium are called *androgynous* or *hermaphroditic.* Other terms widely used are *hermaphrodite*, *monoclinous* and *synoecious*. A complete flower is a perfect flower with petals and sepals. A unisexual-reproductive structure is either functionally male or functionally female. In angiosperms this condition is also called *diclinous*, *imperfect* or *incomplete.* Many plants have complete flowers that have both male and female parts, others only have male or female parts and still other plants have flowers on the same plant that are a mix of male and female flowers. Certain plants even have mixes that include all three types of flowers, where some flowers are only male, some female and some both male and female. A few plants also undergo what is called sex-switching, like *Arisaema triphyllum*, expressing sexual differences at different stages of growth. In some arums smaller plants produce all or mostly male flowers and as plants grow larger over the years the male flowers are replaced by more female flowers on the same plant. Other species have plants that produce more male flowers early in the year and, as plants bloom later in the growing season, they produce more female flowers.

Dioecious refers to a species having separate male and female plants. That is, no individual plant of the species produces both microspores and megaspores; individual plants are either male (producing microspores) or female (producing megaspores). Dioecy is a rare sexual system in flowering plants, occurring in only 4–6% of species (Guttman and Charlesworth 1998; Renner and Ricklefs 1995). In addition, only a minority of these dioecious species have heteromorphic sex chromosomes. Their sex determination system is based on the X/ autosome ratio or on the X/Y ratio (Ainsworth 2000); the Y chromosome is dominant (Charlesworth et al. 2005; Liu et al. 2004). Male genomes consist of an association of three nuclear subgenomes: the autosomes, the X and the Y. The male flower phenotype is not dependent on the presence of the Y chromosomes, but they are necessary for the production of fertile pollen (Negrutiu et al. 2001). The best known cultivated dioecious species for determination of sex expression are: hops (*Humulus lupulus*), spinach (*Spinacia oleracea*), asparagus (*Asparagus officinalis*), carob (*Ceratonia siliqua*), date palm (*Phoenix dactylifera*), fig (*Ficus carica*) and papaya (*Carica papaya*).

The date palm is an important horticultural crop grown mainly in the Middle East and Arabian region (FAO 1984). It is an outcrossed, perennial monocotyledon which is very heterozygous. Researchers have reported that the chromosome number of

date palm is 2n = 2x = 36 (Al-Salih et al. 1987; Beal 1937; Ibrahim et al. 1998). Sexual phenotype is a particularly important problem in dioecious plants that are cultivated for agricultural purposes, as illustrated in studies of the date palm. Date palm is a dioecious species and consequently half of the progeny will be male and half female, with no certain way to determine at an early stage the gender of the progeny nor fruit or pollen quality prior to flowering. As a dioecious species, date palm has male and female flowers being produced in clusters on separate palms. These flowering clusters are produced with axils of leaves of the previous year's growth. In rare cases both pistillate and staminate flowers are produced on the same spike while the presence of hermaphrodite flowers in the inflorescence has also been reported (Mason 1915). Palms which carry both unisexual and hermaphrodite flowers are known as *polygamous*.

At the maturation stage of the date palm tree, it is easy to identify the male and female flowers. The unisexual flowers are pistillate (female) and staminate (male) in character; they are borne in a large cluster (inflorescence) called a *spadix* or *spike,* which consists of a central stem called a *rachis* and several strands or *spikelets.* Male spathes are shorter and wider than the female ones. Each spikelet carries a large number of tiny flowers: as many as 8,000–10,000 in the female and more in the male inflorescence (Chandler 1958). The male inflorescence is crowded at the end of the rachis, while branches of the inflorescence of the female cluster are less densely crowded at the end of the rachis. These characteristics allow the recognition of the inflorescence's sex before it opens. The male flower is sweetly scented and normally has 6 stamens, surrounded by waxy scale-like petals and sepals. Each stamen is composed of two small yellowish pollen sacs. Farmers determine the quality of male date flowers by their smell and other features.

In the wild, the date palm tree pollinates naturally, but fruit production is low. If one were to depend on insects or wind-aided natural pollination, 50% of the trees should be male, which would make date farming by that method uneconomical. Artificial pollination increases production substantially. The dioecious nature of the date palm necessitates the transfer of pollen from a staminate palm to the pistillate in order to obtain an economically feasible yield. Therefore, this necessitates adoption of a manual pollination process to ensure a rich crop. Traditionally to pollinate a spathe on a female palm, a piece of mature male spikelet is inserted into the female spathe as it splits open and is loosely bound around it. The most important benefit of the manual pollination process is that male flowers from a single tree can be used to pollinate 40–50 female date palms.

Sexual propagation is the most convenient method by which to propagate date palm: seeds can be stored for years, they germinate easily and are available in large numbers. The most obvious drawback is the heterozygous characteristics of seedlings which are related to the dioecious nature of the date palm: half of the progeny are generally male, which produce no fruits, and large variations in phenotype can occur in progeny. Furthermore, no method is known at the present for sexing date palm at an early stage of tree development. It is therefore not possible to eliminate non-productive male trees in the nursery before planting on a field scale.

26.3 Sex Identification by Traditional Means

Sex determination is an important developmental event in the life cycle of all sexually reproducing plants and can be controlled genetically by mechanisms also found in the animal kingdom. Dioecious plants provide a particularly interesting system in which to study the genetics and evolution of sex chromosomes. In dioecious species, sex determination is a complex developmental process ending in the differential expression of stamen and carpel genes in male and female individuals. In date palm, the most significant limiting factor in breeding is the time required for maturation of plants, since the palm tree becomes sex identifiable after 5–7 years (Shaheen 1990). Both male and female palms bear spathes; they appear from the bases of the palm leaves towards the top of the palm. The male blossom is fluffy white and star-shaped. Female blossoms resemble beads on a string (Fig. 26.1).

A rapid and easy method for determining the sex of seedlings is considered to be very important for seed cultivation as well as for speeding up breeding programs. In a dioecious plant such as date palm, the genetics of sex determination are far less understood. However, sex expression in many cases is strongly affected by environmental conditions (light, temperature, nutrients) as well as by applied chemical agents (hormones).

The importance of hormones in sex expression has been studied using two different approaches: exogenous administration of growth regulators to developing plants, and quantitative analysis of endogenous hormones. All plant hormones appear to influence sex expression, but unequivocal roles cannot be attributed to a single hormone due to the disparity of the results in different species. Generally it was found that cytokinin; indoylacetic acid and abscisic acid are higher in male than in female plants (Bracale et al. 1990). Accordingly, the induction of the male organ can be attributed to a disturbance in the regulation of gene expression caused by a hormonal balance variation that can lead to an unusual activation of the inhibited male-related loci in the female flower. The success of this derepression or reactivation event is tightly dependent on a distinct stage in the floral developmental process. Similarly, DeMason and Tisserat (1980) reported a female organ induction by a 2,4-dichlorophenoxyacetic acid (2,4-D) treatment of date palm male flowers. This female organ activation might have resulted from a modified gene expression regulation, due to the hormonal treatment of male flowers. However, the authors reported that no ovules were observed in the carpels of the apparent bisexual flowers obtained. They added that, in some cases, apparently bisexual flowers can occur naturally within female date palm trees.

Normal development of male flowers was described by DeMason et al. (1982), who reported that the early development of staminate flowers is identical through carpel initiation. Then, the pistillate and staminate flowers diverge. Recently, a study to investigate the main factors involved in the process of sex modification of female date palm through *in vitro* hermaphrodism induction was reported (Masmoudi-Allouche et al. 2009). That study demonstrated that female date palm flowers can acquire new physiological characteristics and capacities that are quite rarely observed during wild flower development. The vestigial stamens of female date palm flowers display a new and higher capacity to proliferate under particular

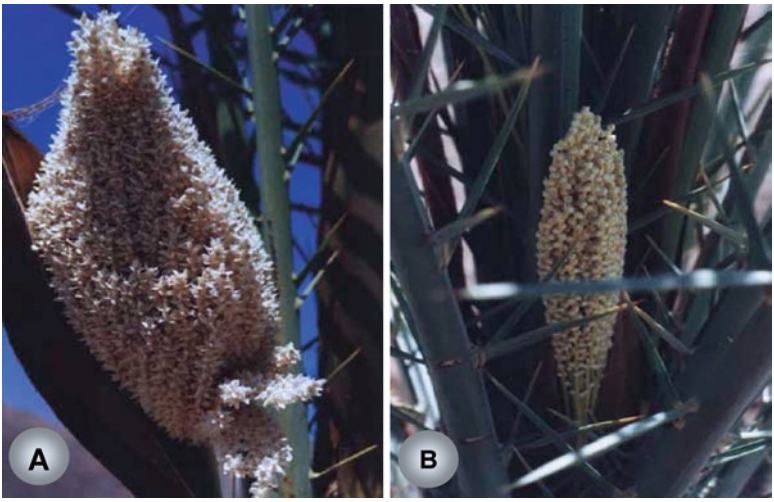

Fig. 26.1 (**a**) Male and (**b**) female inflorescences of date palm (Source: Zaid and de Wet 2002)

in vitro conditions, without blocking carpel development, leading to morphologically typical hermaphrodite flowers.

Recent studies of sex determination in many plant species have been useful in identifying the diversity of genetic and epigenetic factors that are involved in determining the sex of the flower or individual. Amenable to genetic analysis, significant progress has been made toward identifying mutations that affect sex expression. In the genes, an angiosperm tree flower, for example, consists of four stacked components. The components include the sepal area, petal area, male area and female area. The sepal and petal areas are not part of the functional reproductive parts but influence air flow and animal pollinator effectiveness. Genetically, the four flower components are controlled by separate gene sets in three zones within a tree flower: zone 1 = female and male part development controlled by specific gene sets, zone 2 = male part and petal area development controlled by gene sets which assure pollen distribution and pollinator attraction and zone 3 = vegetative zone development controlled by specific gene sets which display, support and protect reproductive parts. In this case, instead of one set of genes conveying gender, trees have developed three unique gene sets which control flower formation and gender expression. Each of these gene sets has been separately driven by many agents to affect efficient reproduction.

Papaya researchers report that the plant's sex-determining region is starting to lose genes for nonsexual traits and to accumulate anomalous DNA. The region has only 62% of the gene density of the rest of the papaya chromosomes. It also shows 28% more rogue genetic elements and nearly triple the amount of DNA with a reversed orientation. There is now no major obstacle preventing isolation of a large set of sex-linked genes from other plants such as those of papaya (Ma et al. 2004). Recent investigations of genes that control the process of sex development reveal that involvement of microRNA in both the sex determination of the male inflorescence and its growth pattern (Banks 2008).

The genetic and physiologic bases (DNA content, frequency of repetitive sequences, genes associated with sex chromosome) of sex expression in *Asparagus officinalis* were studied by Bracale et al. (1990). In addition, stamen and carpel specific messages were searched together with hormonal content of male and female flowers. Major results indicated that gene expression in young male and female flowers is very similar to each other (in agreement with their morphological appearance) and only later on differential expression takes place. On the contrary, important differences in hormonal content between sexes (mainly auxin levels) were observed in meiotic flowers, suggesting that this stage is critical step in the pathway of sex differentiation. In some species, sex expression is under epigenetic control mediated by chromatin modifications of the sex determining regions, including DNA methylation and nucleosomal histone acetylation (Vyskot 1999). There is a growing body of evidence suggesting that epigenetic changes, such as nascent cytosine methylation, are responsible for early stages in the evolution of dioecy and sex chromosomes in all eukaryotes (Gorelick 2003; Gorelick and Osborne 2002). There is only one report suggesting that the date palm is one of the rare monocotyledonous dioecious species that possess heteromorphic X/Y sex chromosomes. In date palms, which like papayas are dioecious with homomorphic sex chromosomes, extra heterochromatin on one of the male chromosomes is believed to determine sex (Siljak-Yakovlev et al. 1996).

Among the many dioecious plant species, only a few have evolved sex chromosomes. Sex determination systems based on heteromorphic X and Y sex chromosomes are particularly interesting to study from both a developmental and an evolutionary perspective. A cytological method based on chromomycin staining which demonstrates the occurrence of sexual chromosomes carrying distinctive nucleolar heterochromatin is described in the date palm which offers, for the first time, the possibility of identifying male and female individuals by simple analysis of root meristems (Siljak-Yakovlev et al. 1996); chromomycin A3 was used to stain root chromosomes, thus identifying subtle differences between the heterochromatin of chromosomes isolated from male and female cells. While useful for sex-typing date palm seedlings, this study also illustrates two other important points in understanding sex determination in dioecious species of plants. First, there are often no obvious cytological or genetic differences between male and female plants; second, it is often difficult to study the genetic or molecular basis of sex determination in many species of monoecious or dioecious agronomically-important plants simply because of their longevity. Otherwise, microsporocytes were examined at late diakinesis and metaphase I of meiosis (Ibrahim et al. 1998). Results showed that in both Samany and Zaghloul date cv. male types, chromosomes tended to pair as bivalents. However, loose bivalents and/or univalents were observed in some pollen mother cells (PMCs) during diakinesis, and cases where more than one bivalent associated with the nucleolus were observed in a few PMCs. Eighteen bivalents at diakinesis indicated that both types were diploid with $2n = 2x = 36$ chromosomes. Otherwise, mitosis was studied in root tip cells of female date cvs. Barhee, Nebut Seif and Succary. Results showed that all three cultivars had a somatic chromosome number of $2n = 36$, and chromosomal behavior was normal (Aly and Bacha 2000).

26.4 Sex Determination Using Biotechnology

Sex determination is a process that leads to the physical separation of male and female gamete-producing structures to different individuals of a species. In the past, various morphological, histological and genetic approaches have been used to determine the gender of higher plants. A genetic test to distinguish between male and female plants would prove useful because it is impossible to tell the sexes apart by looking at the chromosomes under the microscope, unlike the case with many other species. Most the studies on palm characterization, detection of genetic variation and gene mutation have concentrated on the variation in chromosome number and biochemical diversity. Despite increasing research efforts on number of different plant species, there is relatively little information available on the molecular basis of sex determination and it is even difficult to estimate the numbers of genes involved, particularly as the genes which result in organ suppression are unlikely to be the primary sex-determining genes.

The development of molecular markers holds many promises to plant breeders and geneticists in different areas; such as in varietal identification or fingerprinting, estimation of relatedness between different genotypes, discernment of evolutionary relationships and introgression of Mendelian traits into a population. Marker-based selection; however, is the area where molecular markers could have the greatest impact in plant breeding. Dellaporta and Calderon-Urrea (1993) mentioned that plants offer unique systems through which to study sex determination. Because the production of unisexual flowers has evolved independently in many plant species, different and novel mechanisms may be operational. Hence there is probably not one unifying mechanism that explains sex determination in plants.

Our understanding of the evolution of plant sex chromosomes and sex determination should be advanced by the use of molecular markers and several groups of researchers are searching for them. Advances in our understanding of sex determination will come from analysis of molecular biology genetics and the biochemistry of genes controlling sexual determination in plants. To date, there is increasing effort to develop molecular markers tightly linked to the sex determining locus in the plant genome and to isolate the corresponding genes (Caporali et al. 1996). For any program of sex determination in dioecious species, several points should be studied: (a) identification of sex type at the molecular level of the DNA fragments bearing the sex controlling genes which will be achieved by searching genetic markers linked to the sex genes, (b) identification of stamen and carpel specific genes, (c) understanding the process of sex differentiation at the biochemical and physiological level.

Early selection of young seedlings could enhance breeding programs and generate experimental male and female genetic stocks, but no cytogenetic protocol exists for sex determination in an immature date palm. Molecular markers can be effectively utilized to diagnose and select a genotype based on linked DNA markers, long before the phenotype is apparent. This is particularly important in date palm given its long juvenile phase. Despite its major status as a cultivated tree crop, little information is

available on the genetics and molecular genetics of date palm. Until now, there has not been identified a single gene for ergonomically important traits. Isolation of male-specific cDNAs from developing flower buds or reproductive organs has not yet led to discovery of sex determining genes, probably because sex determination occurs very early in flower development, so the genes identified are controlled in response to sex, rather than the controlling loci.

The region containing the sex determining loci must initially have been fully homologous between the two alternative chromosomes. One goal of plant sex chromosomes studies is therefore to test for homology. Both X- and Y-linked markers are now being discovered in plants, with and without heteromorphic sex chromosomes (Harvey et al. 1997; Mandolino et al. 1999; Polley et al. 1997; Testolin et al. 1995; Zhang et al. 1998). Most markers are, however, anonymous, and cannot tell us which X-linked loci have homologues on the Y chromosomes and which do not. The introduction DNA-based markers in date palm will help in developing a sex specific marker. Developing such a marker would allow early determination of sex in palm offshoots at the seedling stage which can help to speed up breeding programs. The cosegregation of the molecular marker and a trait of interest, in progeny segregating for this trait, is an indication of linkage between them. This marker could then be used for selection instead of morphological characters.

Molecular analysis can predict the superior heterotic combination within a set of genotypes. This is helpful in accelerating a breeding program in date palm, which requires many years before flowering. Molecular techniques are extensively used in constructing genetic maps, marker-based selection, cloning useful genes and fingerprinting in several plant species such as tomato and potato, as well as in weed-like *Arabidopsis.* In palms, it could be possible to screen for a desirable genotype at the seedling stage by using marker-based selection strategy. The same method can be used to distinguish between male and female date palms before flowering. The most general approach to achieve this objective is the initial construction of a complete linkage map (Gebhardt and Salamini 1992).

Selection of desirable palms (male or female) can be carried out based on biochemical and molecular markers. Although molecular cloning approaches have not yet identified primary sex determining genes in any dioecious plant species, a range of molecular markers linked to sex have been generated. These markers have either arisen from genetic mapping programs or from research aimed at finding sex-linked markers for agronomically-important dioecious species. In some plants such as *Silene latifolia*, *Cannabis sativa*, *Phoenix dactylifera* and *Rumex acetosa*, therefore, it is not surprising that male-associated markers are relatively abundant.

In dioecious plants where sex chromosomes have not been identified, markers for maleness indicate either the presence of sex chromosomes which have not been distinguished by cytological methods or that the marker is tightly linked to a gene involved in sex determination. Female-associated molecular markers have been described in *Actinidia* (Harvey et al. 1997) and *Salix viminalis* (Alstrom-Rapaport et al. 1998). These may arise as a consequence of close linkage with a female sex determining gene or may indicate a sequence on the X chromosome inherited from the male parent. *Salix viminalis* is unlikely to have sex chromosomes and probably

has a two-locus epistatic system. Moreover, a number of research groups have used subtraction techniques of either cDNA or genomic DNA in attempts to isolate sex determining genes from *Silene latifolia*. Differential screening of a subtracted cDNA library enriched for male-specific sequences enabled nine male enhanced cDNA sequences (Men-1 to -10) to be isolated (Scutt and Gilmartin 1998; Scutt et al. 1997). To date, two groups of researchers have reported randomly amplified polymorphic DNA markers that are highly specific for males and hermaphrodites but absent in females of papaya (Deputy et al. 2002; Urasaki et al. 2002).

The various molecular markers linked to sex include Randomly Amplified Polymorohic DNA (RAPDs), Restriction fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphism (AFLPs) and microsatellites; all are powerful techniques, which have been developed for molecular analysis of plant genome. In this respect, ALFP has been used to initiate a genetic map of date palm (El-Kharbotly et al. 1998). Five cultivars, i.e. Bunarenga, Damous, Fardh, Khalas and Khenizi and pollen of male palm coded BN-96, DN-96, Fr-96, KL-96, and KN-96 amended by the Rumais Agriculture Research Station, Sultanate of Oman, were used to establish F_1 populations. Data taken from 3-week old seedlings showed that the populations were segregating with 1:0, 1:1 or 3:1 ratios for erect and slanting, respectively. The erect and slanting leaf was controlled with a simple genetic factor following Mendelian inheritance. The AFLP fingerprinting for both parental clones was obtained with 32 primer combinations. Three primer combinations (E-AAC, M-CAG; E-AAG, M-CAT and E-ACG, M-CAA) showed few bands in the male parents while few bands were observed in the female parent using the combinations of E-ACG, M-CAA. The polymorphism between the two parents ranged from 4% to 55% depending on the primer combination. This study showed the possibility of using the AFLP technique to characterize the date palm genome. A map can be constructed based on the pattern of the segregation of ALFP generated bands. The authors mentioned that linkage groups can be constructed based on the cosegregation of different bands.

RFLP have several advantages over morphological and isozyme markers and are currently contributing greatly to the construction of detailed genetic maps. The level of allelic variation of RFLP markers in plant population is much greater than that of morphological or isozyme markers. Furthermore, RFLP markers usually behave in a codominant manner, are apparently free of epistatic effects and are developmentally stable. A detailed RFLP map could lead to the identification of new genetic markers that are tightly linked to sex-determining genes. In this respect, a preliminary genetic map of the dioecious species of *Asparagus officinalis* has been constructed on the basis of Restriction fragment Length Polymorphisms (RFLP) and isozyme markers (Restivo et al. 1995). One isozyme and three RFLP markers were assigned to the sex chromosome. Otherwise, RFLP markers have been used to distinguish between the sexes in *Asparagus* (Biffi et al. 1995). Microsatellite banding patterns have been shown to be sex-specific in *Carica papaya*, (GATA) (Parasnis et al. 1999). In *Phoenix dactylifera*, the sexes can readily be distinguished by cytological examination of interphase nuclei in root tip cells. Cells from male plants carry two fluorescent blocks of unequal intensity while female cells carry two equal blocks (Siljak-Yakovlev et al. 1996).

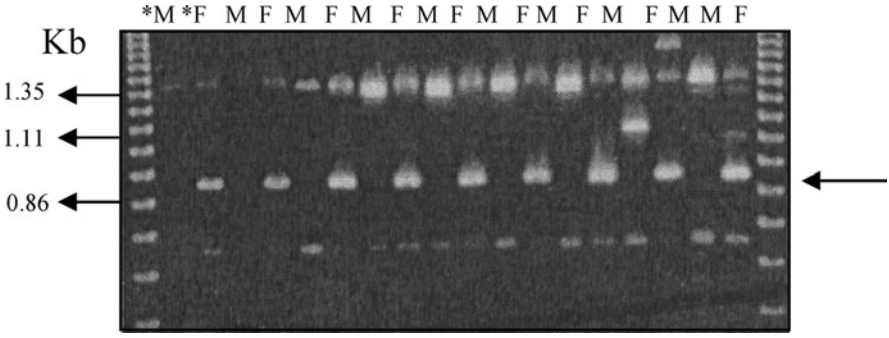

Fig. 26.2 RAPD banding patterns from pooled DNA from male and female 'Kerman' progeny (*M, *F, respectively) and DNA extracted from 7 each of the male (M) and female (F) individuals comprising the pools using primer OPO08. OPO08945 is indicated with an *arrow* (Source: Hormaza et al. 1994)

RAPD technique has been used extensively in plants for various purposes such as genetic diversity, DNA fingerprinting, classification and phylogenetic studies. A glance at the potential applications of RAPD marker technology reveals its importance as a powerful tool in plant molecular biology which can play an important role in genetics and breeding. RAPD markers have already been used for determining sex by bulk segregant analysis in *Pistacia vera*, *Atriplex garretti*, *Trichosanthes dioica* and *Salix viminalis*. Also, RAPD has been used successfully to create a linkage map (Soundur et al. 1996) and sex determination (Saker and Kuehne 1998) in papaya and (Hormaza et al. 1994) in *Pistacia vera* (Fig. 26.2). In this respect, Saker and Rady (2003) analyzed male and female papaya plants using different classes of molecular markers i.e., isozyme and RAPD in order to identify new polymorphisms as a first step towards developing of a universal molecular marker linked to sex in papaya. Analysis of peroxidase isozyme banding patterns indicated that one peroxidase isomer is peculiar only to male trees. RAPD indicated that one polymorphism DNA fragment detected in banding patterns of male clones was absent in female.

In an attempt to determine the genetic difference between male and female date palms, genome DNA was extracted from leaves of four female cvs. (Deglet Noor, Allig, Kentich, Menakher), a male genotype pollinator T23 and F_1 hybrid. The results of RAPD gave reproducible polymorphic bands with 11 primers from 53 primers used. The RAPD thus was successfully used to differentiate between female cultivars, male and F_1 hybrid (Ben-Abdallah et al. 2000). Soliman et al. (2003) used RAPD technique to compare genetic material from four female date palms and four unknown male Egyptian trees. The banding profiles obtained suggested that two male clones are genetically related to the four female date palm cvs. (Zaghloul, Amhat, Samany and Siwi) ranged from 87.5% to 98.9%. In this respect, identification of some Egyptian date palm males from females varieties using molecular markers was reported (Ahmed et al. 2006). Genomic DNA and RNA were extracted, measured and used as a template to detect genetic relationship and similarities

among four known females (Sakkoty, Malkabi, Bartamoda and Dagana cvs.) and three unknown males of Egyptian date palm based on DNA and RNA technology. Results showed that differential display and RAPD analysis provided a rapid and effective method to detect the genetic relationship and similarities between four males and females of Egyptian date palms.

For early identification of cultivars and tracing genetic diversity among date palm genotypes of different origin, offshoot-derived, male and female plants of cvs. Barhee and Sukkary, seed-derived plants, and two *in vitro* cultures of both of these cultivars were subjected to RAPD analysis (Al-Khalifa et al. 2006). Similarity matrixes show that offshoot-derived male plant of Barhee was 73.6% genetically similar to its female counterpart, while similarity between male and female plants of Sukkary was 43.1%. In the case of seedlings, male and female plants of Barhee were 87.2% similar and those of Sukkary were 62.3% genetically alike.

Recently, sexual embryos of date palm were *in vitro* cultured and molecular analysis was used for early identification of sex type (Bekheet et al. 2008). In that study, the potential of isozymes and RAPD markers in sex identification of *in vivo* grown and *in vitro* differentiated cultures of date palm was investigated. *In vitro* zygotic lines were proliferated from mature and immature zygotic embryos of date palm. Early estimation of sex type of *in vitro* differentiated lines has been realized via the activity levels of two enzymes. A high level of peroxidase activity has been observed in adult and offshoot females. Acid phosphatase and glutamate oxaloacetate enzymes gave a strong difference between male and female date palms. Otherwise, the RAPD technique was used to compare genetic material from male, female and unknown lines of date palm. RAPD analysis showed a relatively close relation between the two females (adult and offshoot) cultures, since they have large number of homologous bands. Although, there was a low relationship between male and female, results of similarity could not confirm a link to sex or estimate the sex type of unknown clones. Moreover, an attempt to identify sex-specific DNA markers for date palm using molecular technique (RAPD and ISSR) has been achieved by Younis et al. (2008).

Four dry date cvs. (Sakoty, Bertmoda, Malkabi, Dagana) and three males (Dagana, Malkabi, Sakoty) recognized as superior date pollinators were used in the study. RAPD analyses gave three positive specific markers for females and two for males in addition to five positive specific markers for males in ISSR analysis. RAPD markers have also been used for identify the desirable traits in palmyra palm (*Borassus flabellifer*) to identify sex and high sap-yielding types (Ponnuswami et al. 2008).

26.5 Conclusion and Prospective

Sex determination in date palm continues to be problematic even with the efforts of researchers in the field. As a consequence, until now there is no reliable molecular method for distinguishing the date palm producing female trees from the male trees before the first flowering, which can occur >5 years after planting. In the date palm, a dioecious mode (separate male and female individuals) and the late initial

reproductive age are major practical constraints for its genetic improvement. Furthermore, it is difficult to identify female cultivars according to their morphological characteristics outside fruiting time. Date palm is not of major economic importance to the most technologically advanced countries in North America and Europe. The major producing area of this crop is located in the Near East and North Africa where technical expertise and infrastructure for advanced molecular genetics research are poor. Moreover, there are few research groups which have been involved in the date palm breeding programs. Therefore, comparatively little work has been done on early sex determination of date palm using molecular genetics approach. However, the available data open a new window for identification of a molecular marker linked to sex in date palm. Therefore, in order to solve this problem, a lot of information still needs to be collected and the genome of the date palm must be studied in more detail.

References

Ahmed M, Soliman S, Elsayed E (2006) Molecular identification of some Egyptian date palm males by females varieties (*Phoenix dactylifera* L.) using DNA markers. J Appl Sci Res 2:270–275

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

Al-Khalifa NS, Khan FA, Askari E, Hadi S (2006) *In vitro* culture and genetic analysis of male and female date palm. Acta Hort 725:653–661

Al-Salih A, Al-Najjar NR, Al-Mashdani AN (1987) A study on the chromosome number of two specific female date palm cultivars. Date Palm J 5:134–143

Alstrom-Rapaport C, Lascoux M, Wang YC et al (1998) Identification of a RAPD marker linked to sex determination in the basket willow (*Salix viminalis* L.). J Hered 89:44–49

Aly M, Bacha M (2000) Chromosome characteristics of three date palm cultivars (*Phoenix dactylifera* L.). Sultan Qaboos Univ J Sci Res Agric Sci 5:11–14

Banks JA (2008) MicroRNA, sex determination and floral meristem determinacy in maize. Genome Biol 9:204.1–204.3

Beal J (1937) Cytological studies in the genus *Phoenix*. Bot Gaz 99:400–407

Bekheet S, Taha H, Hanafy M, Solliman ME (2008) Morphogenesis of sexual embryos of date palm cultured *in vitro* and early identification of sex type. J Appl Sci Res 4:345–352

Ben-Abdallah A, Stiti K, Lepoivre P, du Jardim P (2000) Date palm (*Phoenix dactylifera* L.) cultivar identification using random amplified polymorphic DNA (RAPD). Cah Agric 9:103–107

Bendiab K, Baaziz M, Brakez Z, Sedra MH (1993) Correlation of isoenzyme polymorphism and Bayoud-disease resistance in date palm cultivars and progeny. Euphy 65:23–32

Biffi R, Restivo F, Tassi F et al (1995) A restriction fragment length polymorphism probe for early diagnosis of gender in *Asparagus officinalis* L. HortScience 30:1463–1464

Bracale M, Galli MG, Longo C et al (1990) Understanding sex differentiation in *Asparagus officinalis*: genetical and physiological approaches. Acta Hort 271:423–431

Caporali E, Carboni P, Nicoloso L et al (1996) Sex determination and differentiation in the dioecious species *Asparagus officinalis* L. Plant Biosyst 130:79–81

Carpenter J (1979) Breeding of date palm in California. Date Grow Inst Rep 54:13–16

Chandler W (1958) Evergreen orchards. Lea & Fabiger, Philadelphia

Charlesworth D (1985) Distribution of dioecy and self-incompatibility in angiosperms. In: Greenwood PJ, Slatkin M (eds) Evolution-essays in honour of John Maynard Smith. Cambridge University Press, Cambridge, pp 237–268

Charlesworth D, Charlesworth B, Marais G (2005) Steps in the evolution of heteromorphic sex chromosomes. Heredity 95:118–128

Dellaporta SL, Calderon-Urrea A (1993) Sex determination in flowering plants. Plant Cell 5:1241–1251

DeMason D, Tisserat B (1980) The occurrence and structure of apparently bisexual flowers in the date palm, *Phoenix dactylifera* L. (Arecaceae). Bot J Linn Soc 81:283–292

DeMason D, Stolte K, Tisserat B (1982) Floral development in *Phoenix dactylifera*. Can J Bot 60:1437–1446

Deputy JC, Ming R, Ma H et al (2002) Molecular markers for sex determination in papaya (*Carica papaya* L.). Theor Appl Genet 106:107–111

El-Kharbotly A, El-Mardi M, Al-Saadi N, Al-Maharuki Y (1998) Towards the construction of a genetic map of date palm using the Amplified Fragment Length Polymorphism technique (AFLP). In: The first international conference on date palm. Al-Ain, March 8–10, pp 194–207

FAO (1984) Production yearbook. Food and Agriculture Organization of the United Nations, Rome

Gebhardt C, Salamini F (1992) Restriction fragment length polymorphism analysis of plant genomes and its application to plant breeding. Int Rev Cytol 135:201–237

Gorelick R (2003) Evolution of dioecy and sex chromosomes via methylation driving Muller''s ratchet. Biol J Linn Soc 80:353–368

Gorelick R, Osborne R (2002) Inducing sex change and organogenesis from tissue culture in the endangered African cycad *Encephalartos woodii* (Cycadales, Zamiaceae). S Afr J Sci 98:114–117

Guttman D, Charlesworth D (1998) An X-linked gene with a degenerate Y-linked homologue in a dioecious plant. Nature 393:263–266

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

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

Ibrahim AMF, El-Kobbia AM, Kitat FM, Abd El-Kawy NM (1998) Cytological studies on date palm. I. Chromosomal behaviour during meiosis of two date palm (*Phoenix dactylifera* L.) male types. Alex J Agric Res 43:237–246

Komai F, Shikazono N, Tanaka A (2003) Sexual modification of female spinach seeds (*Spinacia oleracea* L.) by irradiation with ion particles. Plant Cell Rep 21:713–771

Liu ZY, Moore PH, Ma H et al (2004) A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature 427:348–352

Lloyd D (1975) The transmission of genes via pollen and ovules in gynodioecy angiosperms. Theor Popul Biol 9:299–316

Lloyd D, Bawa K (1984) Modification of the gender of seed plants in varying conditions. Evol Biol 17:255–338

Ma H, Moore PH, Liu Z et al (2004) High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. Genetics 166:419–436

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

Masmoudi-Allouche F, Chaari-Rkhis A, Kriaa W et al (2009) *In vitro* hermaphrodism induction in date palm female flower. Plant Cell Rep 28:1–10

Mason S (1915) Botanical characters of the leaves of the date palms used in distinguishing cultivated varieties, Bulletin 223:1–28. USDA, Washington, DC

Mulcahy DV, Weeden NF, Kesseli RV, Carroll SB (1992) DNA probe for Y-chromosome of *Silene latifolia*, a dioecious angiosperm. Sex Plant Rep 3:1–3

Negrutiu I, Vyskot B, Barbacar N, Moneger F (2001) Dioecious plants. A key to the early events of sex chromosome evolution. Plant Physiol 127:1418–1424

Okada S, Sone T, Fujisawa M et al (2001) The Y chromosome in the liverwort *Marchantia polymorpha* has accumulated unique repeat sequences harboring a male-specific gene. Proc Natl Acad Sci USA 98:9454–9459

Parasnis AS, Ramakrishna W, Chowdari KV et al (1999) Microsatellite (GATA)M reveals sex-specific differences in papaya. Theor Appl Genet 99:1047–1052

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

Ponnuswami V, Jagadeesan R, Ramesh Kumar A et al (2008) Genetic relationship and diversity in palmyrah accessions based on rapid markers. Am Euras J Sust Agric 2:165–171

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

Restivo FM, Tassi F, Biffi R et al (1995) Linkage arrangement of RFLP loci in progenies from crosses between doubled haploid *Asparagus officinalis* L. clones. Theor Appl Genet 90:124–128

Saker M, Kuehne T (1998) RAPD marker and RAPD-based marker (SCAR) distinguish male from female papaya plants *in vitro*. Third Arab Conference on modern biotechnology and areas of applications in the Arab world, Cairo, 14–17 Dec 1998

Saker M, Rady M (2003) Employment of molecular markers for identification of male and female papaya plants. J Gen Eng Biotechnol NRC 1:85–97

Scutt CP, Gilmartin P (1998) The men-10 cDNA encodes a novel form of proline-rich protein expressed in the tapetum of dioecious *Silene latifolia*. Sex Plant Rep 11:236–240

Scutt CP, Li Y, Robertson SE, Willis ME, Gilmartin PM (1997) Sex determination in dioecious *Silene latifolia* (effects of the Y chromosome and the parasitic smut fungus (*Ustilago violacea*) on gene expression during flower development). Plant Physiol 114:969–979

Shaheen M (1990) Propagation of date palm through tissue culture: a review and an interpretation. Ann Agric Sci Ain Shams Univ Cairo Egypt 35:895–909

Siljak-Yakovlev S, Cerbah M, Sarr A et al (1996) Chromosomal sex determination and heterochromatin structure in date palm. Sex Plant Rep 9:127–132

Soliman S, Bahy A, Mohamed Morsy M (2003) Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. Afr J Biotechnol 2:86–87

Soundur N, Manshardt R, Stiles J (1996) A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. Theor Appl Genet 93:547–553

Testolin R, Cipriani G, Costa G (1995) Sex segregation ratio and gender expression in the genus *Actinidia*. Sex Plant Rep 8:129–132

Urasaki N, Tokumoto M, Tarora K et al (2002) A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.). Theor Appl Genet 104:281–285

Vyskot B (1999) The role of DNA methylation in plant reproductive development. In: Ainsworth CC (ed) Sex determination in plants. BIOS Scientific Publisher, Oxford, pp 101–120

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

Zaid A, de Wet PF (2002) Botanical and systematic description of the date palm, In: Zaid A, Arias-Jimenez EJ (eds) Date palm cultivation, Chapter 1. FAO, Rome, pp 1–47

Zhang YH, DiStilio VS, Rehman F et al (1998) Y chromosome specific markers and the evolution of dioecy in the genus *Silene*. Genome 41:141–147