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III. MICROSPOROGENESIS AND POLLEN FORMATION

IN THE DATE PALM, *Poenix dactylifera L.*

ABSTRACT

The status of chromosome number and karyotype analysis in the date palm, Phoenix dactylifera L. has been recently indicated in the 4th International Date Palm Conference (FIDPC), held in Abu Dhabi, UAE, during the period 15-17 March 2010, and some of the important literature on the issue was presented (1). The present article deals with the phenomenon of microsporogenesis and pollen formation in the Anthophyta, with some emphasis made on the process, as it occurs in microsporocytes in the anthers from staminate flowers obtained from male spadices of date palm. The present article provides a complementary knowledge to that indicated in the previous article, and may represent a valuable source for studying the haploid chromosome number in date palm and in establishment of the Karyotype in this socio-economically important plant crop i.e., the Blessed Tree, Phoenix dactylifera L.

INTRODUCTION

Cytologists associated with Anthrophyta (flowering plants) are aware of the fact that microsporocytes in meiosis and cells in pre- and post-meiotic mitoses, may be secured by choosing flower buds of specific lengths. Such cytological and growth correlation has been well demonstrated in Brassica (Person, 1933), Lillium longflorum (Erickson, 1948), Nicotiana (Al-Ani , 1964), in few species of Datura (Al-Ani,1969; Al-Ani and Al-Okaily 1990; Al-Ani et al., 2008), and in the egg plant (Joodi, N. A. et. al., 1996).

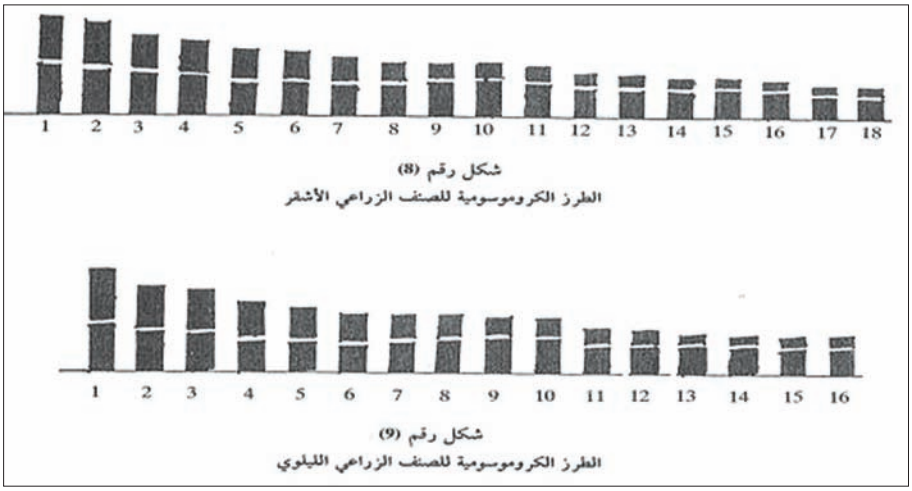
The date palm is a dioecious plant, as the male flowers occur in one plant and the female flowers on another separate plant. The male or female flowers are formed in a special type of inflorescence called a spadix. From the taxonomic view point, the spadix is a compound spike, and each

strand in the bunch represents a spikelet. The whole inflorescence is enveloped by a thick leathery wall called the spathe which completely surrounds the inflorescence and protects the flowers from mechanical damage. It also provides suitable micro-environment to developing flowers. Not until the spadix becomes mature, that the spathe cracks open and the flowers in spadix become visible. Morphological and developmental aspects of male and female spadices of the date palm are described in Al-Bakr (1972), Zaid and Arias (2002) and Shabana et.al, (2006).

Spadix length, color, number per palm tree, time at which it splits open, and other anatomical and morphological characteristics are essentially determined by genetics. However, nutrition and environmental conditions, especially temperature, also have marked influence on these spadix features.

Generally, some female and also male cultivars are known to be early, intermediary or late in ripening. The number of male spadices ranges from 10-30 spadix per palm per annum; and in females between 0-25 per adult trees per annum. It is commonly known that the spadices do not develop and ripen synchronously, as they usually ripen one after the other over a period that may extend for 40 days or so in some cultivars,(Shabana et al., 2006). Such nonsynchronous characteristic feature of spadix ripening is considered advantageous in agricultural practice and for cytogenetic and breeding investigations, as they permit successful pollination over a wide range of time in each season.

Usually, male spadices are longer and wider as compared to female ones. The number of branches in a male spadix is greater than that in a female spadix,(almost double), and as many as 300 strands may be found per male spadix. But the average strand length in males does not exceed 25 cm, while

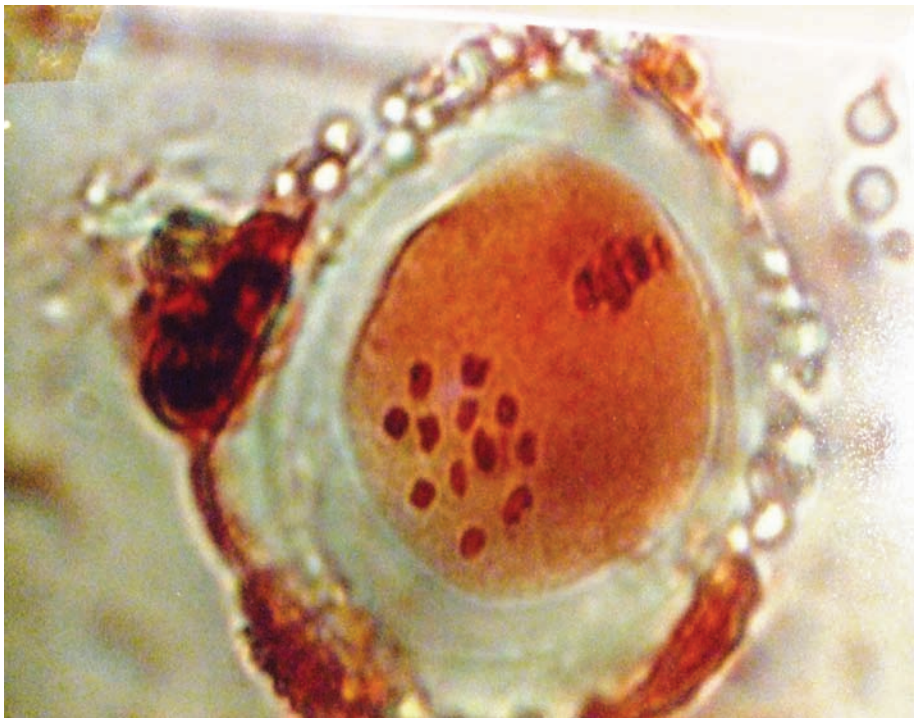


Caryotype of Date Palm . After Al Salih and Al Rawi, 1987

Trillium, Microsporocytes in M1 showing 5 bivalents

the strands in female spadix may range between 10-45 cm. As to the number of flowers in the spadix, it is usually greater in the males and may approach 10,000 male flowers per spadix (Zaid and Irias, 2002).

Male flowers have six stamens each, and each stamen consists of a filament that carries an anther in which pollen grains are formed. A single male spadix may produce 15-35 gm of pollen grains, and their viability and durability vary with the cultivar and storage conditions. Each gram of pollen contains about 2 million pollen grains (Shabana et.al.,2006). This means that the number of pollen grains per spadix is approximately 30-70 millions.



Microsporocytes in Datura at M2 showing 12 bivalents. After Al-Ani and Al- Okaily (1990)

HISTOGENESIS OF MALE SPADIX

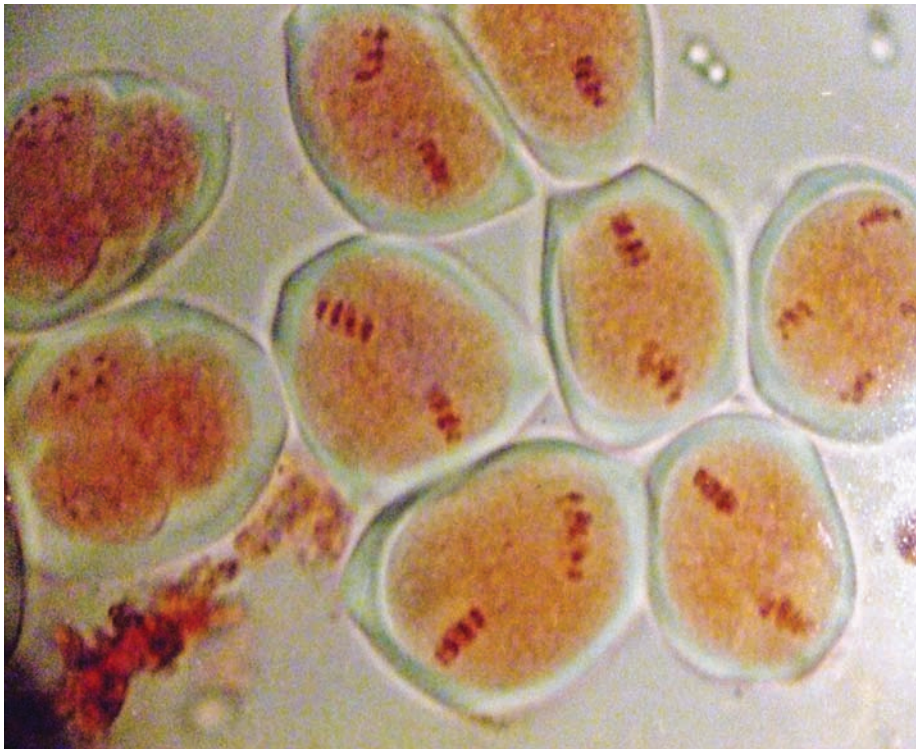
As in other angiosperms, the shoot apex of date palm follows the Tunica-Corpus Theory (Fahn, 1977; El-Jarrah and Al-Ani 1981;Esau, 1986; Al-Ani & Najeeb, 1988, Al-Ani et al., 2008).

The shoot apex of Phoenix dactylifera contains two Tunica layers (T I and T II) and a corpus (c). In cross section, the anther consists of two lobes joined together by a sterile tissue called the connective. Each lobe, in turn, consists of 2 locules called the pollen sacs. Thus, each anther contains 4 pollen sacs. It is in the pollen sacs, which represent the microsporangia, that the pollen grains are initiated. In early stages of anther development, primary sporogenous tissue appears in the center of each pollen sac, and soon becomes surrounded by a definite layer called the tapetum. Histogenesis of primary sporogenous tissue revealed that this tissue is derived from the second layer of tunic (T II). The cells of primary sporogenous tissues, prior to meiosis, contain a diploid number of chromosomes (2n) and they soon function as microspore mother cells, also called pollen morther cells (PMCs) or microsporocytes. Each PMC, by meiosis, gives rise to a group of four haploid cells called the microspores, which remain associated together for some period of time.

As long as the microspores remain associated together in groups of 4s after meiosis, they are called microspore tetrads. The four microspores in each tetrad will later become released from the microsporocyte wall and each becomes independent of the other members of the tetrad. At this stage, each microspore is uninucleate and has the haploid (1n) number of chromosomes. The nucleus of each microspore will then undergo a mitotic division (called the first post-meiotic mitosis) to produce a binucleate pollen grain. One of the two nuclei of a pollen grain is called the generative nucleus while the second is called the tube nucleus (or vegetative nucleus).

The generative nucleus will undergo mitosis, called the second post-meiotic mitosis, to produce 2 male gametes (2 sperm), while the tube nucleus will never divide again. The latter nucleus controls the apical growth of pollen tube and becomes degenerated soon after the pollen tube discharges its contents into the embryosac. The two male gametes which contain 1n chromosome number each, will both function in the double fertilization characteristic of angiosperms, producing a 2n zygote; and a 3n primary endosperm nucleus. The 2n (or diploid) zygote develops to the embryo of the seed, while the 3n primary endosperm nucleus gives rise to the triploid (3n) endosperm. The endosperm is a part of the seed, outside the embryo. It represents a polyploid storage tissue which provides nutrition for the embryo and the young developing seedling. In certain plants, e. g. Pisum, Vicia faba (broad beans) and many other angiosperms, the entire endosperm tissue is digested by the developing embryo, and the mature seed lacks the endosperm. Such seeds are called non-endospermic (or ex-endospermic). In other flowering pants such as species of the Gramineae, Palmae and many others, the endosperm tissue is still present in the mature seed. In date palm the endosperm constitutes the major part of the seed and nutritionally supports seed germination and early development of seedling.

As a rule, most storage paranchyma cells have thin walls, and storage materials are present in the cytoplasm. In the endosperm of date palm seed the the parenchyma are surrounded by very thick primary walls rich in hemicellulose, which serves as reserve substance. In Coffea, the endosperm parenchyma also have rather thick primary walls rich in hemicelluloses which are the bases of coffee industry. In some countries date palm seeds



Microsporocytes in Datura at M2. After Al-Ani and Al- Okaily (1990)

(pits) are used to make date coffee. It is surprising that such reserve material was able to perpetuate and supports seed germination of date palm in seeds that are about 2000 years old. In spite of that, the seeds proved to have maintained its totipotency and true-to-typeness over such a long period of time, and to achieve successful seed germination and produce date palm plants.

From the embryogenetic and cytogenetic view points, the process of microsporogenesis is completed by the time the second meiosis has completed and the microspore tetrads have formed. Microgametogenesis, however, begins with the stage of microspores formation until the formation of the three- celled pollen tube.The latter stage of pollen tube represents a mature male gametophyte.

HOMOLOGY

Homology is a term used to denote the anatomical and histogenetic resemblance of structures, organs or tissues. If, for example, two organs have similar pattern of initiation and development, they are said to be homologous. For example, sepals and petals are homologous with the foliage leaf. The histogenesis of all these organs indicates that they originate almost solely from the first and second tunica layers (T I and T II), and the contribution of the corpus (C) is restricted to a very minor portion in the core of the leaf base in each. These studies of homology are best supported by periclinal chimeras which indicate marked similarity in the histogenesis of sepals, petals, and foliage leaves (Avery, et al.,1959).

On the same bases, the anther is considered homologous with the leaf, and the whole anther represents a microsporophyll while the pollen sacs represent microsporangia; and these tissues are considered to be homologous with each other.

Similarly, the three-celled pollen tube stage, represents a mature male gametophyte, while the 8- nucleate embryosac represents a mature female gametophyte. For further information about the subject, the reader is referred to the huge information presented by Blakeslee (Avery et.al., 1959), Esau (1965) and in the chapter on chimeras in the revised edition of the Essentials of Plant Anatomy (Al-Ani and Najeeb, 1988). The subject of homology has been supported by suitable periclinal chimeras in a number of flowering plants such as Datura. Periclinal chimeras of the types: (2n, 4n, 2n) or (4n,2n,4n) for example have been found highly valuable in histogenetical investigations; and provided firm support to studies of homology, histogenesis , organogenesis and morphogenesis.

SUITABILITY OF MICROSPOROGENESIS FOR CYTOGENETICS RESEARCH

The process of microsporogenesis and pollen formation takes place in the anther. In the date palm, Phoenix dactylifera L., this process takes place in anthers that belong to flowers present in a spadix of specific length. As in other angiosperms, various stages of microsporogenesis and microgametogenesis may be secured from flower buds of specific lengths. The suitability of spadix for scientific investigations may be inferred from the following characteristics of the spadix and flower buds or flowers which are enclosed within the spathe.

The most important developmental features of the spadix and its contents may be summarized as follows:

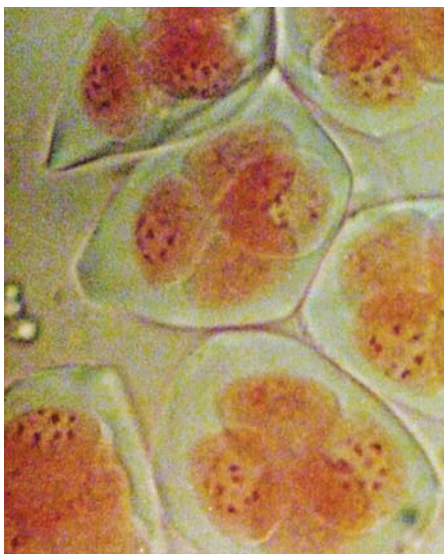
1. The spadix grows exponentially from the earliest stages of its initiation until the spadix approaches its full size , and the logarithmic values of spadix growth in length has a linear, or a straight line mode of growth curve as a function of time.

2. Any stage of meiosis (or

microsporogenesis) as well as pre- and post-meiotic mitoses can be secured from buds at specific spadix length. For example, microsporocytes at metaphase of the first meiosis (MI) may be secured when spadix has emerged to a visible distance of about 15-20 cm from the crown of leaves among which they develop. Such correlation between morphological and cytological events in spadix may vary slightly depending on the cultivar , geographical location and also on environmental conditions. The anthers are approximately 2-3 mm in length when the microsporocytes are in metaphase of the first division of meiosis (MI) and again, this has to be determined for each male cultivar and location of date palm tree.

Although the microsporocytes (PMCs) at this stage of development are relatively small, yet these cells can readily be separated from each other in smearing, and the bivalents are easily stained. (Beal, 1937; Al-Ani & Al-Okaily, 1990 and Al-Ani et.al., 2008).

3. Anther contents belonging to the same flower are highly synchronous, and this is true not only in anthers within the same flower, but also among flowers located at the same level at the main axis of the inflorescence. Thousands of microsporocytes can selectively be obtained in this manner. The phenomenon of synchrony is even more pronounced in stages of long duration such as the prophase of the first division of meiosis (P1) and in the microspore tetrad stage at the end of meiosis, where almost all of the cells are at the same stage of development. This feature of spontaneous synchrony in cell population is of a high value in scientific research. Such synchronous population of cells can be utilized especially conveniently in this system for cytogenetic investigations and in studying some physiological and biochemical aspects of meiosis and pre- and post-meiotic mitoses.



Microspore tetrads in Datura. After Al-Ani and Al- Okaily (1990)

As early as the 1950s the first induced synchronous division in mass cultures of animal cells was performed by Scherbaum and Zeuthen. Tetrahymena was subjected to a series of thermal shocks, as a result of which a high percentage of cells started to divide synchronously. However, such experimentally induced synchrony in cell population in this ciliate, free-living protozoa has lasted after few generations when the cultures were left under normal temperature (about 37 degrees Celsius). Yet this temporary induced synchrony has stimulated a great deal of research, Zeuthen (1971); Peter (1979); Masui and Wang (1998). Experimentally induced synchronization was found to produce some over-sized cells which may have some impacts on biochemical, physiological and ultrastructural aspects of cell metabolism. During microsporogenesis, however, such concerns are avoided, as cell population in this system is spontaneously synchronous and all biological phenomena are going on under normal genetic control without interruption. Such synchrony is highly valuable for karyotype analysis and helps the researcher detect trisomy (2n=1) , tetrasomy (2n=2), double trisomy (2n=1=1), monosomy(2n-1) and other aneuplody.

Furthermore, it helps detect polyploidy by observing the association of homologous chromosomes at late prophase 1 , and their configuration in late diakinesis and in metaphase I. At the latter stage, normal diploid plants of date palm show 18 bivalents. In trisomics condition 17 bivalents are observed, in addition to one trivalent configuration of the homologous chromosomes involved in the trisomic condition..

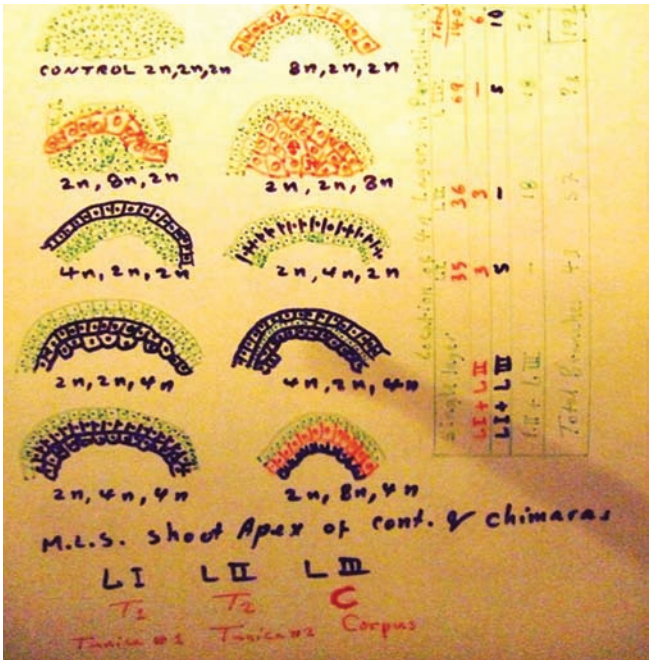
Similarly, all other aneuploidies can be detected by observing the type of configuration of homologous chromosomes at M1.

Regarding Euploidy, one can also detect the degree of ploidy from the cofiguration of the homologous chromosomes association at M1. In triplod plants (3n), for example, metaphase I should reveal 18 trivalents instead of the 18 bivalents normally observed in normal diploid plants. Likewise triploidy (3n), other euploid conditions such as tetraploidy (4n), pentaploidy (5n), hexaploidy (6n) etc, can be detected in the same way in many other plant species, though information on the existence of such polyploidies in date palm are rather scarce, and requires careful cytogenetic investigation.

4. In microsporogenesis, the cytologist deals with the reduced number of chromosomes (1n), especially from late prophase I until microspore tetrads formation and in post-meiotic mitosis. This helps the researcher count the chromosomes with ease, and detect any chromosome anomalies in more certainty as compared to the case when dealing with diploid chromosome complement (2n).

5. The logarithmic values of spadix length represents an excellent time index that helps the researcher determine the duration of meiosis in vivo and to secure

any stage of meiosis or other stages of anther development by utilizing this physiological or developmental time index (Bhojwani and Bhatnagar, 1982, Al-Ani, 1969;A-Ani & Al-Okaily, 1990 and Al-Ani, et.al., 2008). Such investigations should be conducted on various male cultivars of date palm in order to determine the duration of meiosis and to throw light on some physiological and developmental aspects of anther development and pollen grains viability. Furthermore, the logarithm or natural logarithm of spadix length may be used to study the allometric relationships between spadix length and many developmental aspects of the organs enclosed within the spathe.



Periclinal chimera. After Avery, et al., (1959)

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