

Chapter 28

In Vitro Flowering of Date Palm

F. Masmoudi-Allouche, B. Meziou, W. Kriaâ, R. Gargouri-Bouziid,
and N. Drira

Abstract Flowering is a complex process which is regulated by several intricate external and internal factors that make its induction under *in vitro* culture highly sensitive. Recently, *in vitro* flowering has been extensively investigated for many plant species and significant advances in the understanding of this phenomenon have been made. However, with regard to date palm trees, this research area remains virtually unexplored. Accordingly, the present chapter aims to present some observations concerning *in vitro* flowering of date palm, and discusses the possible factors involved in the *in vitro* flowering induction of these plants. It also describes *in vivo* flowering in date palm and the limitations associated with its requirements. The chapter goes on to present the genes controlling the flowering process and to discuss the *in vitro* induction of bisexual flowers in date palm. The chapter concludes by discussing the importance of *in vitro* flowering to date palm propagation and its implications for future programs of early sex determination and genetic improvement via hybridization practices.

Keywords Biotechnology • Micropropagation • Plant tissue culture

28.1 Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious, heterozygous and perennial tree that belongs to the Arecaceae family. The palm family is composed of various combinations of hermaphrodite species, namely *Nannorrhops ritchiana*,

F. Masmoudi-Allouche (✉) • B. Meziou • W. Kriaâ • N. Drira
Laboratory of Plant Biotechnology, Department of Biology, Faculty of Sciences Sfax,
Road Soukra BP. 1171, 3000 Sfax, Tunisia
e-mail: faizamasmoudi@yahoo.fr; besmameziou@yahoo.fr; maswkri@yahoo.fr; drira_riadh@yahoo.fr

R. Gargouri-Bouziid
Department of Biology, Institute for Engineering studies, University of Sfax,
Road Soukra BPW, 3038, Sfax, Tunisia
e-mail: gargouriradhia@yahoo.fr

Sabal palmetto and *Washingtonia filifera*, and other monoecious species, such as *Elaeis guineensis* and *Cocos nucifera*, as well as dioecious ones, including *Borassus flabellifer* and *Phoenix dactylifera*. At the morphological level, four architectural models have been described for the various structures in palms by Holtum, Corner, Shoute and Tomlinson (Halle et al. 1978). According to the Tomlinson model, the date palm produces offshoots at the base of the tree and lateral inflorescences (Tomlinson 1961).

Date palm constitutes a major income-generating activity and food supplying source to millions of people mostly in the arid and warm regions of the Middle East and North Africa, which are favored by suitable dry subtropical and high temperature. Unfortunately, however, the cultivation of date palm is troubled by several internal and external problems worldwide. Among the most disturbing are the complexities involved in the cultivation process and the exposure to damaging diseases that menace growth, productivity and production yields.

Because date palm is slow flowering and fruiting, a major problem is to identify the sex of the samplings at an early stage, which in turn would allow for the cultivation of adequate number of productive female trees with only a minimal number of male trees.

Apart from the obvious problems emanating from the slow rates of growth and the imperceptibility of sex before flowering, date palm is also prone to severe selective pressures exerted by several pernicious diseases. Prominent among these are the bayoud vascular disease, which is caused by *Fusarium oxysporum* f. sp. *Albedinis* and has prevailed in North Africa for more than a century, and the brittle leaf disease, which emerged in Tunisia in the early 1980s and still is unknown as to its source (Triki et al. 2003). The severity of these diseases and consequent losses in production yields vary from year to year depending on the prevailing conditions.

In order to preserve the date palm and to improve its growth conditions, several propagation techniques have been developed and used with varying degrees of efficiency and success. Conventional propagation methods, to start with, are based on vegetative multiplication using offshoots. Although often leading to seedlings that are closely similar to the mother plant, these techniques have often been reported to be very slow and risky for it can cause the dissemination of infectious diseases. Another popular technique for date palm propagation is through sexual reproduction. This seed-dependant strategy actually generates a very heterogeneous population of male and female individuals in almost equal proportions (50% each). The sex of the date palm remains indiscernible until flowering, which may not occur until the plant reaches the age of 6–8 years.

Since the traditional improvement programs are based on hybridization, the Nixon research group envisaged a selection process for date palm based on repeated pollinations of some elite cultivars, such as Deglet Noor, by males derived from the same cultivars with the hope of adopting multiplication by seedling without losing the essential characteristics during the successive multiplication generations (Nixon 1959; Nixon and Furr 1965). After two generations, however, and due to the slowness of the method and the long vegetative cycle of this species, this strategy was abandoned.

To overcome these limitations and in an attempt to regenerate date palm cultivation in Morocco, as well as to control the damage caused by the bayoud disease, attempts have been envisaged to create new and useful cultivars based on controlled crosses, using resistant male and female plants. The cultivars regenerated were of poor quality. This line of research was also abandoned because of the lack of sufficient information on date palm genetics and difficulties pertaining to its biology (e.g. dioicy, long vegetative cycle) (Saaidi 1979).

Recent research seems to have paid special attention to the development of viable alternative propagation methods that can best surmount the shortcomings and inadequacies of the propagation techniques mentioned above. Of particular interest, *in vitro* culture techniques have been described to offer quite handy opportunities to produce date palm cultivars of high quality and with resistance to biotic stress. Moreover, classic breeding requires sexual hybridization, and the flowering process is crucial for the selection programs. Within this context, *in vitro* flowering seems to present a quite promising candidate that would open new pathways in genetic improvement and selection programs of this species.

In date palm, flowering has long been considered a complex process regulated by intricate internal and external factors and its induction under *in vitro* culture has often been reported to present an extremely sophisticated venture. In fact, only a few studies have so far been carried out to investigate this phenomenon in date palm. In this context, *in vitro* flowering has been successfully induced in vegetatively-propagated plantlets of different date palm cultivars (Masmoudi-Allouche et al. 2009). The highest flower induction rates were obtained through alternating between hormone-free and -rich media under different light/dark conditions. This sex induction constitutes a novel system that may allow for early sex determination and to explore the *in vitro* flowering in relation to the photoperiodic requirements in date palm. The conversion into inflorescences involved the entire apical vegetative meristem of the plantlet without affecting its phyllotaxis. A change in the architectural model of date palm was induced. Such *in vitro* flowering, producing typical female flowers, allows a significant reduction in plant cycle and can, therefore, be considered a valuable tool for future genetic improvement and selection programs in date palm. Moreover, *in vitro* induction of bisexual flowers in date palm has been achieved. The vestigial stamens of female flowers display a new and high capacity to proliferate under particular *in vitro* conditions leading to morphologically typical hermaphrodite flowers. Such hermaphroditism control can provide new prospects for improving the understanding of the genetic mechanism involved in sex organ development in date palm.

28.2 *In Vivo* Flowering

Normal date palm flowering follows an annual cycle consisting of four major phases. Date palm inflorescence development occurs in winter (Northern Hemisphere) while the vegetative growth undergoes a rest phase, during which the growth of all

Table 28.1 Date palm biological cycle (Northern Hemisphere) (Saaidi 1979)

Month	Vegetative development	Reproductive organ development
January	Vegetative growth rest	Inflorescence growth
February		Inflorescence emergence/pollination
March	Vegetative growth	Fruit maturation
April	departure	
May		
June	Full vegetative activity	Fruit size increase
July		
August		Maturation
September		
October	Growth slow down	Maturation/harvest
November		
December	Vegetative growth rest	Inflorescence initiation

the organs is blocked. In spring, when the soil temperature exceeds 12°C, the vegetative development takes place again. In summer, the plant undergoes full vegetative activity. In autumn, the date palm shows a slow growth period; it is the season that characterizes fruit maturity and harvest (Table 28.1) (Saaidi 1979).

The date palm inflorescence is composed of an axis, the spadice (or peduncle), which divides into branches (pedicels) that bear the flowers. The latter are unisexual and develop on distinct plants. Each inflorescence is enclosed in a large bract (spathe) whose shape constitutes a sex characteristic. The inflorescences derive from the development of the inflorescencial buds located at the leaf axils (Bouguedoura 1991).

Date palm flowers are trimeric and unisexual. In addition to the 3 sepals and 3 petals of the flowers, the male flower is characterized by an androecium composed of 6 stamens formed in 2 whorls of 3 stamens each. The male flower also contains 3 vestigial carpels. The female flower, however, develops a gynoecium composed of 3 free carpels; it also contains 6 vestigial stamens (staminodes) (Masmoudi-Allouche et al. 2009).

In fact, the *in vivo* requirements that govern floral induction, initiation and development are not yet fully known and elucidated. Further research is particularly needed to determine the photoperiodic prerequisites of date palm *in vivo* flowering and floral induction.

28.3 *In Vitro* Flowering

The phenomenon of *in vitro* flowering, though proved as a valuable approach that can be integrated into breeding programs for date palms, has received much less attention than other domains of *in vitro* research. The few reports currently available

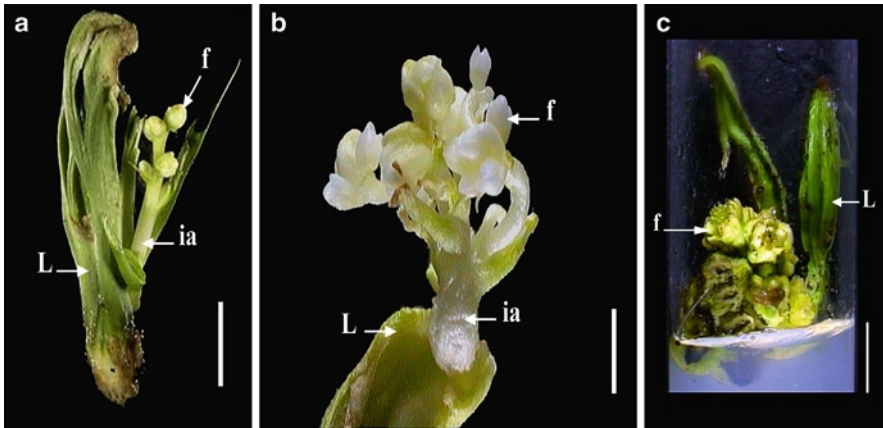


Fig. 28.1 *In vitro* flowering of date palm plantlets. (a) Inflorescence reduced to a single floral axis, (b) Ramified inflorescence (typical inflorescence), (c) Inflorescence reduced to a single flower. The photo shows two flowers induced on two plantlets cultured together in the same test tube. *L* leaf, *ia* inflorescencial axis, *f* flower. Scale bars: $10^4 \mu\text{m}$

in the literature include the work conducted by Ammar et al. (1987), investigating the sexual induction of young seedlings obtained from the germination of Deglet Noor seeds. They indicated that both male and female flowers were induced in 5-month-old seedlings on 16 h day at 28°C using BAP (6-benzylaminopurine), IAA (Indole acetic acid) and glucose or sucrose. Masmoudi-Allouche et al. (2010) also achieved *in vitro* flower induction in 1-year-old *in vitro*-grown date palm plantlets (Fig. 28.1), regenerated from bud cultures via organogenesis using juvenile leaves taken from the offshoots (Drira 1983) of several date palm cultivars.

In fact, the scarcity of data in this area of research is attributed to the complex and multifaceted factors governing and affecting *in vitro* date palm flowering.

28.3.1 Factors Involved in *In Vitro* Flowering Induction

The literature presents a wide array of studies that have been conducted on multiple plant species and that focused on a variety of factors involved in *in vitro* flowering. These factors include light (Heylen and Vendrig 1988), photoperiod (Floh and Handro 2001), pH of the medium (Jumin and Ahmad 1999), lipids (Groenewald and Westhuizen 2004), putrescine and silver nitrate (Bais et al. 2000, 2001), and nutrients (Franklin et al. 2000; Wang et al. 2001; Zhang 2007). Several studies have also focused on the effect of plant growth regulators on the *in vitro* flowering process in some species. The requirement of cytokinin for the growth and development of flower bud has, for instance, been reported in both monocots and dicots (Wang et al. 2001; Zhang 2007; Zhong et al. 1992; Zhou et al. 2004). The effect of BA (6-benzyladenine) on early *in vitro* flowering has also been reported for different

plant species (Hee et al. 2007; Jumin and Ahmad 1999; Jumin and Nito 1996; Sim et al. 2008; Wang et al. 2001). Other reports described the combined effect of BA and other hormone species on the induction and stimulation of *in vitro* flowering (Galoch et al. 2002). Similarly, Tang et al. (1983), Das et al. (1996) and Wang et al. (2002) have reported on *in vitro* flowering using BAP (Benzylaminopurine) and GA3 treatments. The influence of cytokinins has also been reported in several studies (Kachonpadungkitti et al. 2001; Taylor et al. 2005; Zhang and Leung 2000). Likewise, the effect of GA3 alone in the induction of *in vitro* flowers in gerbera (*Gerbera jamesonii* Adlam) has been reported (Ranasinghe et al. 2006). Last but not least, the individual and combined effects of abscisic acid (ABA) and proline on *in vitro* flowering in *Vigna aconitifolia* have been described by Saxena et al. (2008).

28.3.1.1 Effect of Plant Growth Regulators and Physico-Chemical Conditions

The significant effect of cytokinins on *in vitro* flowering has been extensively described and documented in the literature (Saritha and Naidu 2007; Wang et al. 2001). The combined effect of auxin and cytokinin on *in vitro* flower induction has also been reported in several studies (Handro 1983; Wang et al. 2002). Likewise, Taylor et al. (2005) reported that phytohormones affected flowering by mediating growth changes within the apical meristem and that cytokinins, in particular, played a key role in the initiation of mitosis and the regulation of cell division and organ formation. Similarly, Galoch et al. (2002) suggested that in the case of morning glory (*Pharbitis nil*), the floral transition involved a multifactorial signaling system, including the photoperiodic conditions, the endogenous phytohormone concentrations and the exogenous phytohormone application, with different phytohormones acting sequentially to trigger various steps in the flowering process.

Furthermore, Ammar et al. (1987) and Masmoudi-Allouche et al. (2010) reported on the combined effect of cytokinin and auxin on date palm flowering. Ammar et al. (1987) described the *in vitro* flowering in date palm seedlings using BAP and IAA treatment. In this study, the cultures were composed either of the cotyledonary petioles enclosing the embryos or the isolated embryos. The experiments were conducted on basal MS (Murashige and Skoog 1962) media supplemented with agar (7 g·l⁻¹) and either sucrose (30 g·l⁻¹) or glucose (50 g·l⁻¹). The phytohormones IAA and BAP were added to the basal medium either alone or in combination at different concentrations. All the cultures were maintained in a growth chamber at 16 h photoperiod at a constant temperature of 28 ± 1°C. The authors reported that both male and female inflorescences were produced in the case of embryos enclosed in the cotyledonary sheath on a medium containing glucose (50 g·l⁻¹), IAA (1 mg·l⁻¹) and BAP (1 mg·l⁻¹). Interestingly, inflorescence production was reported to have always been preceded by leaf initiation and to occur only in the absence of root development. In the case of excised embryo culture, on the other hand, male and female inflorescences were produced axillary to leaves, in branches. Such *in vitro* flower formation was reported to have occurred on a medium supplemented with sucrose

Table 28.2 Composition and concentrations of plant growth regulators of M1, M2, and M3 culture media

Culture medium	M1	M2	M3
Nutrient solution	MS + ½ QL major salts	MS + ½ QL major salts	MS
Plant growth regulators (µM)	NAA: 2.68 BAP: 4.44 Kin: 4.64 IPA: 5.28	No growth hormones	

(30 g·l⁻¹), IAA (0.1 mg·l⁻¹) and BAP (10 mg·l⁻¹) and to be always preceded by the development of very small leaves with reduced limbs.

In a study by Masmoudi-Allouche et al. (2010), *in vitro* flower induction experiments were conducted on basal MS (Murashige and Skoog 1962) media supplemented with sucrose (50 g·l⁻¹), adenine (0.03 g·l⁻¹), L-glutamine (0.1 g·l⁻¹) and agar (8 g·l⁻¹). In some cases, half-strength major salts of Quoirin and Lepoivre's nutrient solution (QL, Quoirin and Lepoivre 1977) were added to the basal MS medium (M1 and M2, Table 28.2). The plantlets were alternatively subcultured every 30 days on M1 (hormone-rich medium) and M2 medium (hormone-free medium) or on M1 and M3 medium (hormone-free medium) (Table 28.2).

The assays performed in Masmoudi-Allouche et al. (2010) involved three sets of experimental protocols. While the first experimental set was exposed to light (15 µM·m⁻²·s⁻¹) at 16 h photoperiod, the second was exposed to continuous dark and the third to sequential 4-week light/dark treatments. Each treatment was applied to 24 explants of each cultivar (1 explant = 1 replicate) and the number of plantlets that showed initiation and development of inflorescences was recorded on a monthly basis.

Moreover, the experimental battery of tests performed by Masmoudi-Allouche et al. (2010) used a combination of cytokinins and auxin (M1) to evaluate their effect on flowering induction. The findings generated from the data analysis revealed that, in the absence of hormonal alternation (M1/M2 or M1/M3), no floral induction was allowed by all the light/dark conditions tested. However, regardless of the light/dark conditions used, the application of a hormonal treatment corresponding to the monthly subculture alternation of M1/M2 or M1/M3 media allowed the induction of an important flowering capacity for the different varieties tested.

More accurately, when the plants were cultured under light/dark alternations and subjected to alternative subcultures of M1/M2 media, the cumulative 1-year flowering rates reached 48% for the Arichti cv., while they did not exceed 25% for the Deglet Noor and Boufeggous cvs. and 10% for the Bousthammi Noir cv. However, when the light/dark alternation conditions were combined with a hormonal alternation between M1 and M3 media, the best flowering rates were obtained with the Arichti cv. whose cumulative 1-year flowering rate increased to reach 58% (Table 28.3). Furthermore, when the hormonal alternation of M1 and M3 media was coupled with light (16 h photoperiod) and continuous dark conditions, the flowering percentages for the Arichti cv. were 51% and 53%, respectively. These flowering

Table 28.3 The effect of various physico-chemical treatments on the flowering percentage of date palm cultures of Arichti cv. that underwent a monthly alternative subculture on M1/M2 and M1/M3 media

Treatment condition	Cumulative 1-year flowering percentage
<i>M1/M3 alternation</i>	
Light	51
Dark	53
16 h light/dark alternation	58
<i>M1/M2 alternation</i>	
Light	25
Dark	40
16 h light/dark alternation	48

percentages were actually much more important when compared to those produced in light (25%) and dark (40%) conditions in the case of a monthly alternation between M1 and M2 media (Table 28.3).

The authors suggest that the acquisition of an important flowering capacity occurred in plantlets that were subjected to sudden change from hormone-rich to -free media. This flowering ability increased when the plants were subjected to a concentration deviation involving both a medium that was rich in hormones and MS and QL nutrients (M1), and a medium that was hormone-free and that contained only the MS major salts (M3).

28.3.1.2 The Culture Period Effect

In a study by Masmoudi-Allouche et al. (2010) date palm plantlets were subcultured for 45 instead of 30 days and were submitted to a hormone alternation between M1 and M2 or M3 media (Table 28.2). The percentages of flowering explants obtained were even much more significant. For the Arichti cv., the cumulative 1-year flowering rate increased from 25% to 43%. Moreover, although the pH of the culture media was first adjusted to 5.8, it decreased during the culture period. In fact, after 30 and 45 days of culture, this decrease was of a 0.4 and a 1.8 pH unit, respectively. This phenomenon revealed that active exchanges occurred between the culture media and the tissues of the explant during the culture period and, might, therefore, have created a stress condition due to the increase of the subculture period.

The effect of a chemical stress on the enhancement of *in vitro* flowering has previously been suggested by Thorpe (1980) for the common chicory (*Cichorium intybus*) subjected to hydrous stress. Similarly, Neelu (1997) showed that flowering induction can be achieved by chemical stress using a medium containing 100 mM NaCl and an appropriate hormonal composition. These stress conditions favored the induction of *in vitro* flowering on date palm tissues, which consequently allowed the expression of new potentials that were not expressed under natural conditions. In fact, the highest flower induction rates achieved through the joint alternation between hormone-rich and -free media together with the light/dark changes could be attributed to an amplification in terms of stress condition and tissue disturbance, which might have ultimately generated a high ability to undergo a reproductive morphogenesis and flower formation, as previously suggested by Tang et al. (1983).

Table 28.4 The best cumulative means over 1-year flowering percentages of date palm plantlets from the different varieties tested when cultured under light/dark alternations and subjected to alternative subcultures of M1/M3 media

Date palm cultivar	Cumulative 1-year flowering percentage	Time of initial flowering response (month)
Arichti	58	1
Deglet Noor	32	3
Boufeggous	28	3
Bousthammi Noir	13	5

The values represent the cumulative 1-year percentages of monthly means of 24 replicates each

28.3.1.3 The Genotype Effect

Very little data are available in the literature regarding the critical effect of genotype on date palm flowering. To date, and to the authors' knowledge, only one report, by Masmoudi-Allouche et al. (2010), explored the genotype effect on date palm *in vitro* flowering using several date palm cvs., namely Deglet Noor, Arichti, Bousthammi Noir and Boufeggous. The offshoots that were investigated were collected from Deglet Noor and Arichti cvs. in the south of Tunisia and from Bousthammi Noir and Boufeggous cvs. in Morocco. An important flowering capacity was observed for the different cultivars. However, the percentages of the neo-formed flowers and the initial response of the *in vitro*-grown plantlets were tightly dependent on the cultivar of the date palm used (Table 28.4). The *in vitro* flowering efficiency observed in this study varied with the date palm varieties used, which is in agreement with the results described by Kenza and Chlyah (1998) that correlated *in vitro* differences in plant tissue responses with genotype effect.

28.3.2 Histological Analysis of the *In Vitro* Flowering Process

Histological studies are useful in providing information concerning structural changes during the course of flowering and to determine the optimal timing and conditions for attaining maximal induction outcomes. Such histological examination is very scarce in the literature. In one of the few currently available histological analyses that examined the floral initiation process in palm date, Masmoudi-Allouche et al. (2010) identified the morphological changes that the *in vitro* plantlet apical buds underwent to generate a floral state (Fig. 28.2a, b). In this study, the samples were fixed in Svaloff Navashine solution (chromic acid 0.5%, glacial acetic acid 5%, formaldehyde 15% and ethanol 5%) at room temperature, washed with running water for 24 h, dehydrated in ethanol solution series (50°–100°) and then immersed in xylene-ethanol baths as described by Masmoudi-Allouche et al. (2009). Paraffin inclusion was subsequently performed and 10- μ m-thick sections were made using a rotary microtome. The sections obtained were stained with a Regaud ferric hematoxylin solution (Regaud hematoxylin solution 10%, glycerol 10%) (Masmoudi-Allouche et al. 2009) and then observed under a light microscope.

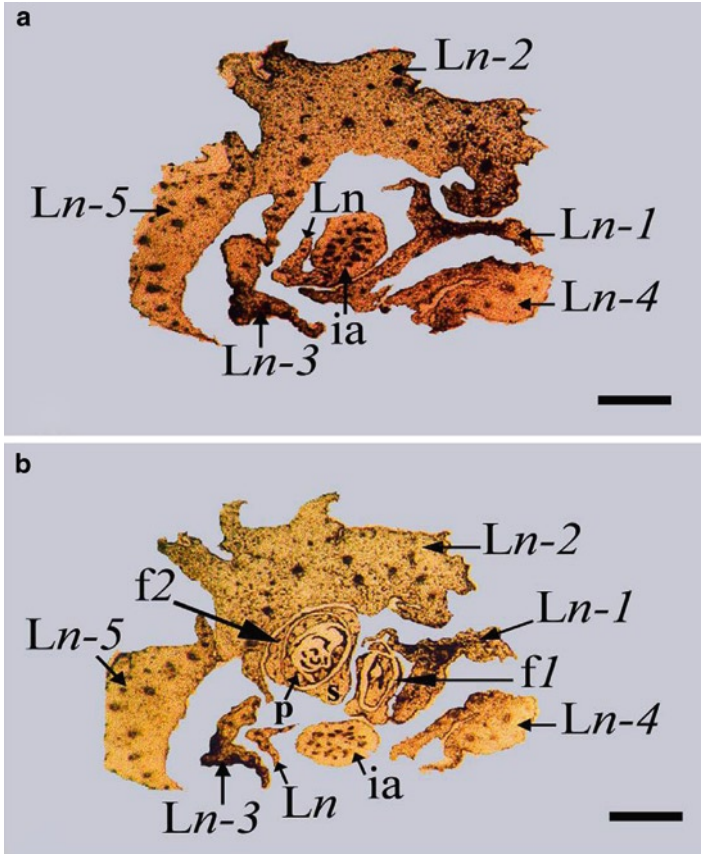


Fig. 28.2 Transverse sections on two different levels of the apical bud of date palm vitroplant cv. Arichti that have reversed to the floral growth. (a) Transverse section on the basal level of the apical bud showing the aspect of the last leaf structures formed at the time of the reversion to the floral state. (b) Transverse section on the upper level of the apical bud showing the development of the inflorescences on which two flowers were initiated. ia: inflorescences axis ; f1: flower 1 in longitudinal section ; f2: flower 2 in transverse section showing, from the external to the internal part, the 3 welded sepals (S), the 3 free petals (P) and the primordia of the reproductive organs of the flower. Ln: leaf of rang n (last leaf that was formed before the reversion process). Ln-1, Ln-2, Ln-3, Ln-4 and Ln-5: leaves of rang n-1, n-2, n-3, n-4 and n-5, respectively. Scale Bars: 1,200 μ m

The findings presented in Masmoudi-Allouche et al. (2010) revealed that the apical vegetative meristem of the vitroplants, which were submitted to the flower inductive treatment, continued to form leaf primordia (Fig. 28.2a) according to the usual phyllotaxis of 2/5 index (Bouguedoura 1980). It was then noted to undergo a sudden change, without any transition, into a structure of inflorescences type (Fig. 28.2b). In contrast, Bernier et al. (1981) claimed that the flower evocation in the shoot meristem was accompanied by a number of events that affected the growth habit of the plant including changes in phyllotaxis and leaf shape.

Fig. 28.3 Date palm female flower resulting from *in vitro* culture in longitudinal section. *s* sepal, *p* petal, *c* carpel, *st* staminode, *o* ovule. Scale Bar: 666 μ m

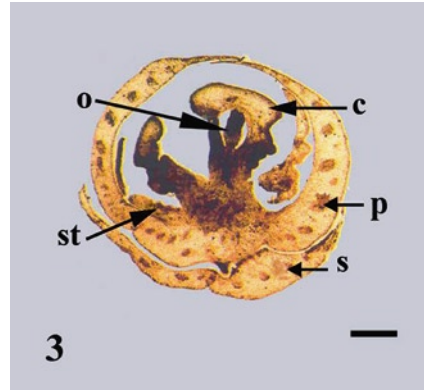
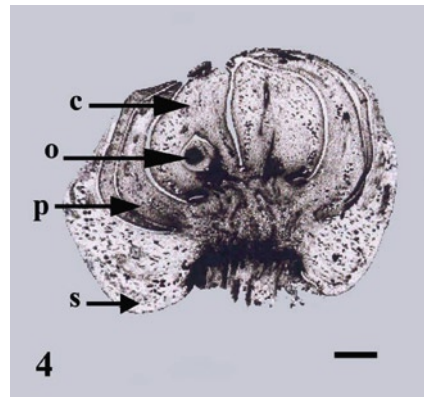


Fig. 28.4 Mature *in vivo* female date palm flower in longitudinal section. *s* sepal, *p* petal, *c* carpel, *o* ovule. Scale bar: 500 μ m



Starting from a female plant material, the authors obtained flowers of the same sex. These *in vitro* female flowers were morphologically similar to those formed *in vivo*. The histological examination of longitudinal sections that was performed on *in vitro* and wild type date palm flowers showed that the *in vitro* flower structures (Fig. 28.3) were similar to those of the natural female ones (Fig. 28.4). In fact, both types of flowers were globular and consisted of three fused sepals, three free petals, six staminodes (vestigial stamens) and three carpels that harbor ovules. This confirmed the mature state of both flower types.

28.3.3 Architectural Model in Relation to *In Vitro* Flowering

Architectural analysis methods have greatly increased our understanding of plant structure and development and have helped construct the architectural models of

several plant species. With regards to date palm, four architectural models have been defined (Tomlinson 1961). Of special interest, the results of Masmoudi-Allouche et al. (2010) revealed that, contrary to the natural flowering development, the *in vitro* neo-formed inflorescences were completely uncovered, i.e. lacking a spathe (inflorescence envelope). The authors attributed this distinct morphogenesis to the inflorescencial ontogenetic mechanism. In fact, under *in vivo* conditions, date palm flowering is pleoanthic for, according to the Tomlinson model (Tomlinson 1961), the inflorescences evolve from the development of lateral buds located at the leaf axils, and the bract situated at the axillary position of the inflorescencial bud develops into a spathe. However, under *in vitro* conditions, the first leaf axils of the plantlets are empty (devoid of lateral buds), as is the case of plants that derive from seedlings (Bouguedoura 1980). In that particular study, the *in vitro* flowering was terminal (hapaxanthic), resulting from the development of the apical bud that was devoid of any bract, which consequently gave rise to uncovered inflorescences.

The results of Masmoudi-Allouche et al. (2010) are in agreement with those of Ammar et al. (1987) who induced sexuality in date palm seedlings and suggested another model corresponding to the neotenous development of this tree as an herb. The latter suggested that the neoteny interpretation cannot be made only when considering a variation in the original architectural model.

Unlike African oil palm (*Elaeis guineensis*) and coconut (*Cocos nucifera*), whose architectural structures conform to the Corner model, the date palm has an architectural structure that conforms to the Tomlinson model (Tomlinson 1961), and this is due to this species' capacity of ramification. Accordingly, only date palm can be vegetatively propagated using its basal axillary buds (Drira 1983). Bouguedoura (1980) and Drira and Benbadis (1985) defined the relationship between the developmental stage of plants issued from offshoots and the nature of the axillary productions that differentiate. The authors indicated that at the first phase of its development, the date palm produces axillary buds that are both vegetative and inflorescencial. The latter are, however, abortive. It is the development of the vegetative buds only that permits a basal ramification. The development of the inflorescencial buds takes place later at the leaf axils.

Considering the abovementioned data (Bouguedoura 1980; Drira and Benbadis 1985), Ammar (1987) and Benbadis et al. (1985) reported that the multiple expressions of the neoteny make the identification of the architectural model essential. These authors suggest that the neotenic plants were different from the fundamental model since they produce both lateral and terminal inflorescences. In fact, in date palm plants issued from the *in vitro* development of excised embryos, the neoteny was expressed in lateral inflorescences (pleoanthic). These inflorescences may be attributed to the developmental stimulation of inflorescencial buds, which are normally abortive. Concerning the neoteny that occurred in plants issued from the *in vitro* development of embryos at the onset of germination, the authors indicated that it was terminal, and therefore conforming to the Holtum model (Halle et al. 1978).

Furthermore, and in a previous study by Drira and Benbadis (1985), hormonal treatment performed at precocious stages of floral differentiation was reported to induce other morphogenetic programs into the floral meristem, leading to the emergence of axes from the staminodes and the carpels of the flower. Masmoudi-Allouche et al. (2010),

on the other hand, reported that the use of combined hormonal and physico-chemical factors induced the reduction of the inflorescences system, which became limited to the differentiation of either a single flower or a unique axis carrying many flowers.

Taken together, the findings reported by Drira and Benbadis (1985) and Masmoudi-Allouche et al. (2010) confirm the hypothesis of Nozeran (1954) that the trimeric flower of date palm corresponds to one group which is more ramified but highly contracted by the phylogenesis. Its original structure is, therefore, completely masked. This compacted group can appear only accidentally and in particular situations, such as *in vitro* culture conditions.

28.3.4 Photoperiodic Requirements of *In Vitro* Flowering

In a study by Masmoudi-Allouche et al. (2010), the authors explored the mechanism of *in vitro* flower induction control in relation to the photoperiodic requirements in date palm. Such *in vivo* photoperiodic requirements for date palm flowering are not yet fully known. Nevertheless, under *in vitro* conditions, flowering induction was obtained by varying the nature and concentration of the hormonal factor in both continuous dark and 16 h light photoperiod exposure. These data provide additional evidence confirming that date palm flowering occurred in both long-day (16 h photoperiod) as well as short-day conditions. Similarly, *in vivo* date palm flowering usually lasts (Northern Hemisphere) from March to May but, in some cases, can extend from January to the end of September, including both short and long-day conditions. These observations provide further evidence that date palm is able to flower, whatever the photoperiod is, as long as a sufficient photosynthetic activity is properly available. Under the *in vitro* culture conditions presented in the mentioned study, the availability of organic nutrients in the medium was sufficient to ensure flowering in continuous darkness.

28.4 Genes Controlling the Flowering Process

Bernier et al. (1999) indicated that the flowering process is controlled by several tens of genes. They also suggested the existence of an inhibitory system that is able to block the expression of floral identity meristem genes controlled by a flowering repressor gene (embryonic flower1, EMF1). The activity of this system induces an obligatory vegetative growth in most plants. Nevertheless, when blocked, the plant would be able to flower spontaneously after germination. The authors added that this gene complex is present in all plants. The characterization of *Arabidopsis thaliana* flowering mutants suggested that a very complex system controls the flowering process in plants.

According to Calonje et al. (2004), the genetic and molecular characterization of the flowering process in different species reveals a conservation of the basic genetic

mechanisms controlling the early stages of flower formation (Ng and Yanofsky 2001; Theissen and Saedler 1999). In monocots, however, *SQUA*-like genes do not seem to be always functional orthologs of their *Arabidopsis* counterparts, based on their relatively large number and expression patterns (Gocal et al. 2001; Schmitz et al. 2000; Theissen et al. 2000; Yu and Goh 2000). All these data suggest that flower initiation and development may involve common regulatory mechanisms to all the angiosperms as well as species-specific mechanisms whose genetic and molecular bases are yet unknown (Calonje et al. 2004).

In fact future research is needed to understand the genes controlling the flowering process in date palm and to analyze their expression during the reproductive organ development. This knowledge may offer a very important system to identify a sex-marker for this dioecious species, particularly at a time when research studies, namely the one conducted by Masmoudi-Allouche et al. (2009), asserted that *in vitro* floral hermaphroditism induction could be successfully achieved. Genetic analysis can, therefore, be conducted on the bisexual, as well as on the normal unisexual female and male, date palm flowers.

28.5 *In Vitro* Induction of Bisexual Flowers

The plant reproductive systems that pattern floral and sexual differentiation can be monomorphic, with a single bisexual flower type, or polymorphic, with two or more flower types. The majority of flowering plants are hermaphroditic, developing perfect flowers that contain both pistils and stamens. Hermaphroditic individuals produce both male and female gametes (Irish and Nelson 1989).

In plants, the understanding of the sex determination system is closely connected with the knowledge of how separate sexes evolved. The widespread view that all flowering plants arose from a common hermaphroditic ancestor (Cronquist 1988) suggests that much of the floral developmental program is common to all species. An early theory (Darwin 1877) claims that the first plant species were hermaphroditic (Ainsworth 2000; Charlesworth 2002; Lebel-Hardenack and Grant 1997; Negrutiu et al. 2001). It suggests that, during evolution, 10% or so of these plants have evolved, via different evolutionary routes, to floral unisexuality as the spatial separation of their flowers generate evolution toward monoecy or dioecy (Ainsworth 2000).

Date palm sexuality follows the dioecy system, which is a rare sexual system in flowering plants, occurring only in 4–6% of the species (Ainsworth 2000; Guttman and Charlesworth 1998; Renner and Ricklefs 1995; Tanurdzic and Banks 2004). In some cases, however, apparent bisexual flowers seem to naturally occur within female date palm trees (Demason and Tisserat 1980). In fact, *in vitro* production of bisexual flowers in date palm species has been reported by Demason and Tisserat (1980), who induced the *in vitro* carpel development in male flowers, and by Masmoudi-Allouche et al. (2009), who induced the *in vitro* stamen development in female flowers.

28.5.1 *In Vitro* Carpel Development in Male Flowers

Demason and Tisserat (1980) described the occurrence of apparent bisexual date palm flowers through a 2,4-dichlorophenoxyacetic acid (2,4-D) treatment of male flowers. They postulated, however, that the staminodes in cultured pistillate flowers did not expand under the culture conditions they used. The apparent bisexual flowers harbored carpels without ovules.

28.5.2 *In Vitro* Stamen Development in Female Flowers

Masmoudi-Allouche et al. (2009) investigated the vestigial stamens (staminodes) of female date palm flowers from different Tunisian date palm cvs. (Deglet Noor, Gondi, Boufeggous, Allig, Mattata and Kentichi) and observed that those stamens displayed a new and high capacity to proliferate under particular *in vitro* conditions and hormonal treatment without blocking carpel development, leading to morphologically typical hermaphrodite flowers (Fig. 28.5). The pollen mother cells isolated appeared in the anther locules obtained and underwent an ordinary microsporogenesis process (Masmoudi-Allouche et al. 2009).

Among the different hormonal combinations tested, only the one including IBA and BAP (Table 28.5), which were added at different concentrations in the MS basal medium, showed an efficient reinitiating of anther development within the female flower (Table 28.5). In fact, a remarkable proliferation of stamens (80–90%) in the female flowers was obtained when the supplemented IBA/BAP concentrations corresponded to 4.92/4.44 μM and 9.84/4.44 μM (media 5 and 4, respectively; Table 28.5). By lowering the IBA/ BAP concentrations to 2.46/2.22 μM (medium 3, Table 28.5), an induction of about 50% was obtained within the cultured female flowers. However, higher hormonal concentrations of 9.84/8.88 μM and 19.68/8.88 μM (media 1 and 2, respectively; Table 28.5) generated a low percentage of hermaphrodite flower production.

The findings of Masmoudi-Allouche et al. (2009) confirm the early theory suggesting that dioecious plants derive from a hermaphrodite ancestor. They support the system reported by Lebel-Hardenack and Grant (1997), which postulated that in many dioecious species, unisexual floral meristems are sexually bipotent and that a change in the level or ratio of endogenous hormones can trigger a switch between the alternative developmental programs of the sex-determining genes. As far as the unisexual flowers are concerned, an abortion or arrest of the carpel primordia in the male flower and the stamen primordia in the female flower occur in a later span of time (Dellaporta and Calderon-Urrea 1994; Kater et al. 2001).

Such hermaphroditism control can provide new prospects and opportunities for the investigation of the *in vitro* self-fertilization process. It can also offer useful tools for further understanding the genetic mechanisms involved in the sex organ development of date palm.

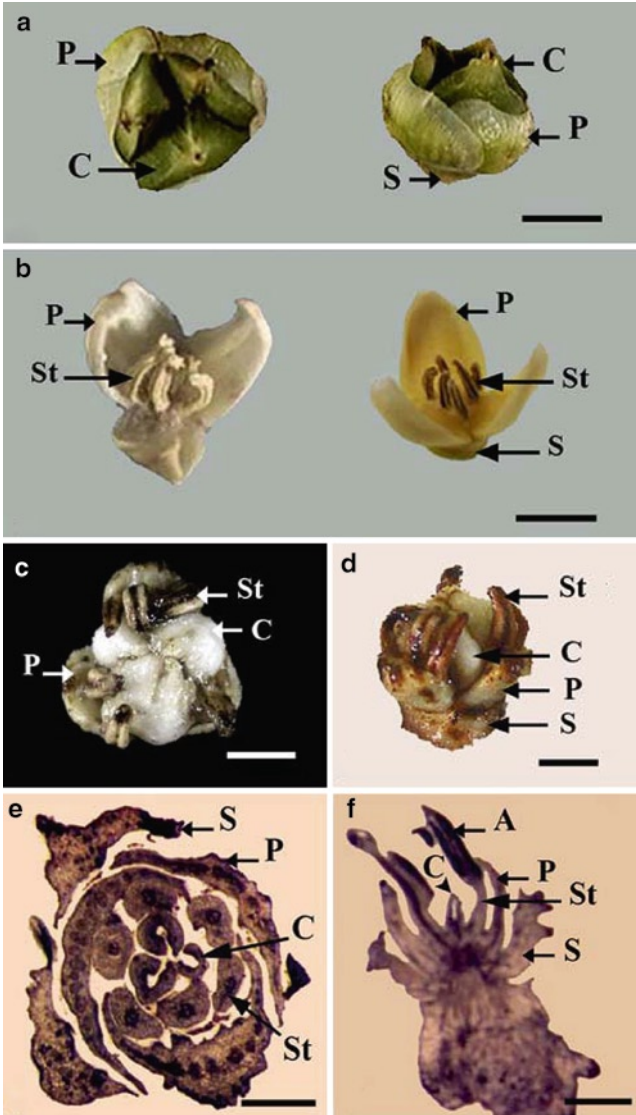


Fig. 28.5 Wild type (a, b) and hermaphrodite date palm flowers differentiated *in vitro* (c–f). *A* wild female date palm flowers; frontal (on the left) and profile (on the right) views. *B* wild male date palm flowers; frontal (on the left) and profile (on the right) views. *C–F* hermaphrodite flower differentiated under *in vitro* conditions ; frontal (c) and profile (d) views, in transverse (e) and longitudinal (f) sections. *S* sepal, *P* petal, *A* anther, *St* stamen, *C* carpel. Scale bars: 1,600 μm (a–d); 500 μm (e, f)

Table 28.5 The percentage of hermaphrodite flowers induced *in vitro* by culturing Deglet Noor female inflorescences on the different tested media for 15 days. About 500 flowers were used for each assay conditions by several repetitions

Medium	IBA (μM)	BAP (μM)	Hermaphrodite flowers, (%)
1	9.84	8.88	5 \pm 0.6
2	19.68	8.88	30 \pm 1.15
3	2.46	2.22	50 \pm 1
4	9.84	4.44	82 \pm 1
5	4.92	4.44	90 \pm 1.5

28.6 Conclusion and Prospects

Date palm has a long juvenile phase that delays their reproductive development by between 6 and 8 years. Although *in vitro* flowering induction has been reported for several plant species, only a few studies have so far been carried out with the aim of accelerating the flowering time of date palm. In fact, the few studies currently available in the literature indicate that *in vitro* flowering induction can be achieved on plantlets regenerated from shoot cultures of different date palm cultivars under particular *in vitro* culture conditions (Masmoudi-Allouche et al. 2010) and also on young seedlings obtained from the germination of Deglet Noor seeds (Ammar et al. 1987). Interestingly, the *in vitro* flowers obtained were histologically and morphologically similar to *in vivo* flowers.

In vitro flowering can be useful to the *in vitro* rejuvenation process which is based on the changes in vegetative characteristics (Hackett 1985; Pierik 1990) and on the flowering ability of cultured shoots (Harada and Murai 1998). These results suggest that the neo-formed flowers have future prospects in developing renovation programs for saving date palm germplasm losing senescence status. *In vitro* flowering will assist to reinitiate the micropropagation process. Furthermore, the significant shortening of the plant cycle through the control of *in vitro* flowering also provides a valuable and promising system for early sex determination. *In vitro* flowering will save time for date palm genetic improvement by using intra- or interspecific crosses. It would help to reveal novel properties and characteristics pertaining to fruit quality and stress resistance.

Moreover, bisexual flowers can be induced *in vitro* through hormonal treatment of female inflorescences leading to high percentages of typical hermaphrodite flowers (Masmoudi-Allouche et al. 2009). Such floral hermaphroditism control will ensure to investigate the *in vitro* self-fertilization process and to identify the sex markers and genes for sex maturation.

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References

- Ainsworth C (2000) Boys and girls come out to play: the molecular biology of dioecious plants. *Ann Bot* 86:211–221
- Ammar S (1987) Contribution à l'étude des potentialités morphogénétiques exprimées en culture *in vitro* par de jeunes plantes de palmier dattier (*Phoenix dactylifera* L.) et des tissus qui en dérivent en culture. Doctoral thesis, Faculty of Science, Tunis
- Ammar S, Benbadis A, Tripathi BK (1987) Floral induction in date palm seedling (*Phoenix dactylifera* L. var. Deglet Nour) cultured *in vitro*. *Can J Bot* 65:137–142
- Bais HP, Sudha GS, Ravishankar GA (2000) Putrescine and silver nitrate influences shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Cichorium intybus* L. cv. Lucknow local. *J Plant Growth Regul* 19:238–248
- Bais HP, Sudha GS, Ravishankar GA (2001) Influence of putrescine, silver nitrate and polyamine inhibitors on the morphogenetic response in untransformed and transformed tissues of *Cichorium intybus* and their regenerants. *Plant Cell Rep* 20:547–555
- Benbadis A, Ammar S, Drira N et al (1985) Evolution du modèle architectural du palmier dattier (*Phoenix dactylifera* L.) liée aux modalités de la multiplication végétative et à l'expression de la néoténie en culture *in vitro*. International colloque on tree, France, pp 14
- Bernier G, Kinet JM, Sachs RM (1981) The physiology of flowering, vol II. Transition to reproductive growth. CRC Press, Boca Raton
- Bernier G, Cremer F, Huijser P (1999) Pourquoi les plantes fleurissent. *Biofutur* 187:22–26
- Bouguedoura N (1980) Morphologie et ontogenèse des productions axillaires du palmier dattier (*Phoenix dactylifera* L.). *C R Acad Sci Paris* 291:857–860
- Bouguedoura N (1991) Connaissance de la morphogenèse du palmier dattier *Phoenix dactylifera* L. Étude *in situ* et *in vitro* du développement morphogénétique des appareils végétatif et reproducteur. Doctoral Thesis, USTHB, Alger
- Calonje M, Cubas P, Martínez-Zapater JM, Carmona MJ (2004) Floral meristem identity genes are expressed during tendril development in grapevine. *Plant Physiol* 135:1491–1501
- Charlesworth D (2002) Plant sex determination and sex chromosomes. *Heredity* 88:94–101
- Cronquist A (1988) The evolution and classification of flowering plants. New York Botanical Garden, Bronx
- Darwin C (1877) The different forms of flowers on plants of the same species. Murray, London
- Das P, Samantary S, Rout GR (1996) Organogenesis and *in vitro* flowering of *Echinochloa colona*. Effect of growth regulators and explant types. *Biol Plant* 38:335–342
- Dellaporta SL, Calderon-Urrea A (1994) The sex determination process in maize. *Sci* 266:1501–1505
- Demason DA, 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
- Drira N (1983) Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) par la culture *in vitro* de bourgeons axillaires et de feuilles qui en dérivent. *C R Acad Sci Paris III* 296:1077–1082
- Drira N, Benbadis A (1985) Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) par réversion, en culture *in vitro*, d'ébauches florales de pieds femelles adultes. *J Plant Physiol* 119:227–235
- Floh EIS, Handro W (2001) Effect of photoperiod and chlorogenic acid on morphogenesis in leaf discs of *Streptocarpus nobilis*. *Biol Plant* 44:615–618
- Franklin G, Pius PK, Ignacimuthu S (2000) Factors affecting *in vitro* flowering and fruiting of green pea (*Pisum sativum* L.). *Euphytica* 115:65–73

- Galoch E, Czaplowska J, Burkacka-Laukajtys E, Kopcewicz J (2002) Induction and stimulation of *in vitro* flowering of *Pharbitis nil* by cytokinin and gibberellin. *Plant Growth Regul* 37:199–205
- Gocal FW, King RW, Blundell CA et al (2001) Evolution of floral meristem identity genes: analysis of *Lolium temulentum* genes related to *APETALA1* and *LEAFY* of *Arabidopsis*. *Plant Physiol* 125:1788–1801
- Groenewald EG, Van Der Westhuizen AJ (2004) The effect of various lipids on flowering of *Pharbitis nil* in *in vitro* culture. *Biol Plant* 48:637–639
- Guttman DS, Charlesworth D (1998) An X-linked gene with a degenerate Y-linked homologue in a dioecious plant. *Nature* 393:263–266
- Hackett WP (1985) Juvenility, maturation, and rejuvenation in woody plant. *Hortic Rev* 7:109–155
- Halle F, Oldeman RAA, Tomlinson PB (1978) *Tropical trees and forests: an architectural analysis*. Springer, Berlin
- Handro W (1983) Effects of some growth regulators on *in vitro* flowering of *Streptocarpus nobilis*. *Plant Cell Rep* 2:133–136
- Harada H, Murai Y (1998) *In vitro* flowering on long-term subcultured pear shoots. *J Hortic Sci Biotech* 73:225–228
- Hee KH, Loh CS, Yeoh HH (2007) Early *in vitro* flowering and seed production in culture in *Dendrobium Chao Praya* Smile (Orchidaceae). *Plant Cell Rep* 26:2055–2062
- Heylen C, Vendrig JC (1988) The influence of different cytokinins and auxins on flower neoformation in thin cell layers of *Nicotiana tabacum* L. *Plant Cell Physiol* 29:665–671
- Irish EE, Nelson T (1989) Sex Determination in monoecious and dioecious plants. *Plant Cell* 1:737–744
- Jumin HB, Ahmad M (1999) High-frequency *in vitro* flowering of *Murraya paniculata* (L.) Jack. *Plant Cell Rep* 18:764–768
- Jumin HB, Nito N (1996) *In vitro* flowering of *Fortunella hindsii* (Champ.). *Plant Cell Rep* 15:484–488
- Kachonpadungkitti Y, Romchatngoan S, Hasegawa K, Hisajima S (2001) Efficient flower induction from cultured buckwheat (*Fagopyrum esculentum* L.) node segments *in vitro*. *Plant Growth Regul* 35:37–45
- Kater MM, Franken J, Carney KJ et al (2001) Sex determination in the monoecious species of cucumber is confined to specific floral whorls. *Plant Cell* 13:481–493
- Kenza L, Chlyah H (1998) Vegetative multiplication of date palms from *in vitro* cultured inflorescences: effect of some growth regulator combinations and organogenetic potential of various cultivars. *Agronomy* 18:573–580
- Lebel-Hardenack S, Grant SR (1997) Genetics of sex determination in flowering plants. *Trends Plant Sci* 2:130–136
- Masmoudi-Allouche F, Chaâri-Rkhis A, Kriaâ W et al (2009) *In vitro* hermaphroditism induction in date palm female flower. *Plant Cell Rep* 28:1–10
- Masmoudi-Allouche F, Meziou B, Kriaâ W et al (2010) *In vitro* flowering induction in date palm (*Phoenix dactylifera* L.). *J Plant Growth Regul* 29:35–43
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Neelu S (1997) *In vitro* completion of vegetative and floral phase of salt - stressed *Brassica juncea* var. BN - 1. *Plant Tissue Cult Biotech* 3:160
- Negrutiu I, Vyskot B, Barbacar N et al (2001) Dioecious plants: a key to the early events of sex chromosome evolution. *Plant Physiol* 127:1418–1424
- Ng M, Yanofsky MF (2001) Function and evolution of the plant MADS-box gene family. *Nat Rev Genet* 2:186–195
- Nixon RW (1959) Pollinization, breeding and selection of date palms. In: *FAO International technical meeting date products and processing*, pp 22–41
- Nixon RW, Furr R (1965) Problems and progress in date breeding. *Date Growers Inst Rpt* 42:2–5
- Nozeran R (1954) Contribution à l'étude de quelques structures florales (essai de morphologie florale comparée). Thèse Doct, Etat Sci Nat Fac Sci, Montpellier

- Pierik RLM (1990) Rejuvenation and micropropagation. *Newsl Int Assoc Plant Tissue Cult* 62:11–21
- Quoirin M, Lepoivre P (1977) Etude de milieu adapté aux cultures *in vitro*. *C R Acad Sci Paris* 281:1309
- Ranasinghe RATD, Abayagunawardana AGNI, Hettiarachchi HIDD et al. (2006) *In vitro* flower induction in Gerbera (*Gerbera jamesonii* Adlam). In: Eighteenth annual congress of the PGIA, pp 16–17
- Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. *Am J Bot* 82:596–606
- Saaidi M (1979) Contribution à la lutte contre le Bayoud, fusariose vasculaire du palmier dattier. Thèse Doct, Univ Dijon
- Saritha KV, Naidu CV (2007) High frequency plant regeneration and *in vitro* flowering of regenerated plantlets of *Spilanthes acmella* Murr – An important threatened bio-insecticide medicinal plant. *Acta Hort* 756:183–198
- Saxena SN, Kaushik N, Sharma R (2008) Effect of abscisic acid and proline on *in vitro* flowering in *Vigna aconitifolia*. *Biol Plant* 52:181–183
- Schmitz J, Franzen R, Ngyuen TH et al (2000) Cloning, mapping and expression analysis of barley MADS-box genes. *Plant Mol Biol* 42:899–913
- Sim GE, Goh CJ, Loh CS (2008) Induction of *in vitro* flowering in *Dendrobium* Madame Thong-In (Orchidaceae) seedlings is associated with increase in endogenous N⁶-(Δ^2 -isopentenyl)-adenine (iP) and N⁶-(Δ^2 -isopentenyl)-adenosine (iPA) levels. *Plant Cell Rep* 27:1281–1289
- Tang AF, Cappadocia M, Byrne D (1983) *In vitro* flowering in cassava (*Manihot esculenta* Crantz). *Plant Cell Tissue Org* 2:199–206
- Tanurdzic M, Banks JA (2004) Sex determining mechanisms in land plants. *Plant Cell* 16:61–71
- Taylor NJ, Light ME, Staden JV (2005) *In vitro* flowering of *Kniphofia leucocephala*: influence of cytokinins. *Plant Cell Tissue Org* 83:327–333
- Theissen G, Saedler H (1999) The golden decade of molecular floral development (1990–1999): a cheerful obituary. *Dev Genet* 25:181–193
- Theissen G, Becker A, Di Rosa A et al (2000) A short history of MADS-box genes. *Plant Mol Biol* 42:115–149
- Thorpe TA (1980) Organogenesis *in vitro*: structural, physiological and biochemical aspects. In: Vasil IK (ed) *Perspectives in plant cell and tissue culture*. Academic, New York, pp 71–111
- Tomlinson PB (1961) Anatomy of the monocotyledons. II. Palmae. Clarendon, Oxford
- Triki MA, Zouba A, Khoualdia O et al (2003) Maladie des feuilles cassantes or brittle leaf disease of date palms in Tunisia: biotic or abiotic disease? *J Plant Pathol* 85:71–79
- Wang S, Tang L, Chen F (2001) *In vitro* flowering of bitter melon. *Plant Cell Rep* 20:393–397
- Wang G, Yuan M, Hong Y (2002) *In vitro* flower induction in roses. *In Vitro Cell Dev Biol Plant* 38:513–518
- Yu H, Goh CJ (2000) Identification and characterization of three orchid MADS-box genes of the *API/AGL9* subfamily during floral transition. *Plant Physiol* 123:1325–1336
- Zhang T (2007) *In vitro* flowering of *Perilla frutescens*. *In Vitro Cell Dev Biol Plant* 43:91–94
- Zhang Z, Leung DWM (2000) A comparison of *in vitro* with *in vivo* flowering in gentian. *Plant Cell Tissue Org* 63:223–226
- Zhong H, Srinivasan C, Sticklen MB (1992) *In vitro* morphogenesis of corn (*Zea mays* L.). *Planta* 187:490–497
- Zhou JH, Zhou JR, Lin BC, Din J (2004) Influences of 6-BA and amino acids on flower formation from cotyledons of cucumber *in vitro*. *Plant Physiol Commun* 6:31–33